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# **Analytical Chemistry: Key to Progress on National Problems**

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**UNITED STATES DEPARTMENT OF COMMERCE**

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**ANALYTICAL CHEMISTRY: KEY TO  
PROGRESS ON NATIONAL PROBLEMS**

Proceedings of the 24th Annual Summer Symposium on Analytical Chemistry sponsored by *ANALYTICAL CHEMISTRY*, ACS Division of Analytical Chemistry, and NBS Analytical Chemistry Division, and held at the National Bureau of Standards, Gaithersburg, Maryland, June 16–18, 1971.

**W. Wayne Meinke and John K. Taylor, editors**

Analytical Chemistry Division  
Institute for Materials Research  
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Washington, D.C. 20234



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## ABSTRACT

This book is the formal report of the proceedings of the 1971 Summer Symposium in Analytical Chemistry held at the National Bureau of Standards, June 16-18, 1971, and cosponsored by the Analytical Chemistry Division of NBS, ANALYTICAL CHEMISTRY, and the American Chemical Society's Division of Analytical Chemistry. It contains six invited papers by subject matter experts comprehensively reviewing urgent research problems for which advanced analytical techniques need to be developed in the important areas of agriculture, air pollution, clinical chemistry and biomedicine, oceanography, solid state research and electronics, and water pollution. Summaries of related discussions by leading analytical chemists serving as panel members or in attendance at the Symposium are included. Hundreds of important analytical problems retarding progress in these important areas are described and extensive references are given to permit a deeper insight into the problem areas. Accordingly this volume should not only stimulate interest in important problems but should provide a valuable guide for highly relevant analytical research for some time to come.

Key words: Agriculture; air pollution; analytical chemistry; biomedicine; clinical chemistry; electronics; oceanography; solid state; water pollution.

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## FOREWORD

The Analytical Chemistry Division of the NBS Institute for Materials Research provides a major national focal point for analytical chemistry through its continuing efforts to encourage meaningful analytical measurements, to exercise leadership in attacking analytical problems of the Nation, and to fill in gaps in critical measurement competences. This Division consists at present of about 100 technical personnel encompassing some 60 different analytical competences from activation analysis and atomic absorption to vacuum fusion and x-ray spectroscopy. These competences are charged with the responsibility for research at the forefront of analysis, as well as for an awareness of the practical sample, be it Standard Reference Material or service analysis.

One important mechanism by which the Division exercises leadership in the "state of the art" of a competence is that of sponsoring special conferences and symposia. Experts from around the world are invited to these conferences to summarize the present status of the many facets of a particular competence. In addition, contributed papers as well as comments of rapporteurs or summaries of panel discussions are often used to help better define this status.

The first such conference sponsored by the Division was a broad one on "Trace Characterization—Chemical and Physical" held October 3-7, 1966 at the new NBS Laboratories at Gaithersburg, Maryland. On June 12-13, 1967 a seminar on a more restricted topic, "Quantitative Electron Probe Microanalysis," was held in these same facilities and on October 7-11, 1968 the Division hosted the 1968 International Conference on "Modern Trends in Activation Analysis." On January 30-31, 1969 a Symposium on Ion-Selective Electrodes was held. The hard-cover proceedings of each of these meetings are available from the Superintendent of Documents, Government Printing Office as NBS Monograph 100, NBS Special Publication 298, NBS Special Publication 312, and NBS Special Publication 314, respectively.

As a continuation of these definitive "state of the art" conferences, the Analytical Chemistry Division was very pleased to join ANALYTICAL CHEMISTRY and the American Chemical Society Division of Analytical Chemistry to host the 24th Annual Summer Symposium in Analytical Chemistry. The theme of this Symposium was "Analytical Chemistry: Key to Progress in National Problem Areas." Over 450 scientists representing a broad spectrum of industrial, governmental, and educational institutions attended, and many others expressed interest in the

proceedings. The format of the symposium provided an excellent opportunity for dialogue between analytical chemists and experts in problem areas of air and water pollution, health, agriculture, oceanography, and solid state research.

This volume contains the six invited papers presented at the Symposium, together with summaries of selected segments of the discussion sessions.

W. Wayne Meinke, *Chief*  
Analytical Chemistry Division

## PREFACE

This book is the formal report of the proceedings of the 1971 Summer Symposium in Analytical Chemistry held at the National Bureau of Standards in Gaithersburg, Maryland, June 16-18. The Analytical Chemistry Division of the NBS Institute for Materials Research joined ANALYTICAL CHEMISTRY and the American Chemical Society Division of Analytical Chemistry in sponsoring this 24th Annual Summer Symposium.

In keeping with its theme—Analytical Chemistry: Key to Progress in National Problem Areas—the Symposium focussed on six very important areas where analytical chemists can play a vital role. These areas included Agriculture, Air Pollution, Clinical Chemistry and Biomedicine, Oceanography, Solid State Research and Electronics, and Water Pollution.

A half-day session of the Symposium was devoted to each of the six subject areas. Leading authorities with broad knowledge of the problem areas were invited to present keynote lectures, summarizing the state-of-the-art of measurements in their fields, and to pinpoint urgent measurement needs for the future. Following the lectures, a panel of analytical chemists, conversant with the measurement problems involved, discussed possible approaches to some of the problems raised by the speakers. Finally, each half-day session ended with a general discussion period in which audience participation was encouraged.

This volume includes the six invited lectures and summaries of the discussion sessions. Many hundreds of important analytical problems retarding progress in important areas are described, with sufficient background information to assist in establishing priority and a rationale for an orderly approach to provide optimum impact in problem solving. Accordingly, we firmly believe that this volume will stimulate interest in many "real-world" problems and provide a valuable guide for highly relevant analytical research for some time to come.

Identification of commercial materials and equipment by the authors in this book in no way implies recommendation or endorsement by the National Bureau of Standards.

An undertaking of the magnitude of the Symposium and this book would not have been possible without the cooperation and assistance of many individuals. The enthusiastic participation of the six invited speakers, the six session chairmen, and the 24 analytical chemists who

served as discussion panel members is deeply appreciated. Many members of the staff of the Analytical Chemistry Division and the National Bureau of Standards served on numerous committees and assisted during the Symposium in various capacities. Particular thanks are given to Bourdon F. Scribner, Richard A. Durst and Philip D. LaFleur for their dedicated efforts in conducting several aspects of the symposium.

The NBS Office of Technical Information and Publications under the direction of W. R. Tilley, and special help from Robert T. Cook, Rubin Wagner, and Mrs. Rebecca Morehouse gave invaluable assistance in many phases of the effort, varying from the initial publicity brochures and program to this final computer-assisted printing and publication of the proceedings.

Within the Analytical Chemistry Division, special thanks are given to Mrs. Barbara Turner and Mrs. Ellen Zimmerman for their untiring effort in typing the coded manuscripts and other material and to Mrs. Joy Shoemaker for preparing the many tables and figures. Particular appreciation is expressed to Mrs. Rosemary Maddock who provided coordination and editorial assistance in the many phases of preparing this book and to James R. DeVoe for developing and providing appropriate interfacing with the Office of Technical Information to expedite the computer typesetting.

November 29, 1971

W. Wayne Meinke

John K. Taylor



**24th ANNUAL SUMMER SYMPOSIUM  
ANALYTICAL CHEMISTRY: Key to Progress  
on National Problems**

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## CHAPTER 1

### EDITOR'S NOTE — —

It is almost forty years since Dr. G. E. F. Lundell presented the following paper before the Division of Physical and Inorganic Chemistry at the 85th Meeting of the American Chemical Society, in Washington, D. C., March 26–31, 1933. The points he makes regarding sampling, practical analysis, accuracy, etc. are as pertinent today as they were then. Readers of this volume will find this paper to be of considerable interest.

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HARRISON E. HOWE, EDITOR

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# The Chemical Analysis of Things as They Are

G. E. F. LUNDELL, Bureau of Standards, Washington, D. C.

**A**T THE outset we might ask the question "Why are chemical analyses made?" The crude analyses of the earliest days were the explorations in an unknown world, the charting of naturally occurring materials and of the simple compounds that were then made by man. Next came the analyses upon which the present structure of chemistry, geology, and many of the other sciences is based. Those were the happy days in which most research workers made their own analyses, and the profession was in high standing. Today, by far the greater number of chemical analyses deal with materials that enter into commerce,

man-made materials for the most part, in which man has outdone his Maker. Moreover, these analyses are usually made by second or third parties who are often entirely divorced from the history of the material or the purpose of the analysis, and who therefore are not only working in the dark but also often have only a monetary interest in the test. The need and importance of chemical analyses are not dying out, for chemical analysis now serves the arts as well as the sciences. It therefore seems appropriate to record some observations on present trends, and on considerations that should not be overlooked.

"The Chemical Analysis of Things as They Are" has been chosen as a subject because so many talks and articles on analytical subjects deal with "The Chemical Analysis of Things as They Are Not." This has been brought about because in the field of analytical chemistry, as in other fields of endeavor, there has been a constant drifting toward specialization. As a consequence, there is an increasing tendency to devote more and more time to determinations which deal with the final act of a chemical analysis, and less and less time to chemical analysis itself—in other words, to consider chemical analysis as dealing with one or two variables instead of the dozen or more that are often involved. This gradual loss of the analytical viewpoint is evident in contemporary articles that purport to deal with chemical analysis.

For example, in a recent article entitled "Determination of Aluminum" it is claimed that good determinations of aluminum can be had by adding lithium chloride, treating with ammonium hydroxide until phenolphthalein turns pink, filtering, washing, igniting, and weighing as the compound  $5\text{Al}_2\text{O}_3 \cdot 2\text{Li}_2\text{O}$ . Not one word is said concerning the behavior of the other elements. To the analyst, it is self-evident that approximately one-half of the elements can be precipitated under these conditions. His chief thoughts on encountering such a reaction would therefore not concern its use for the determination of aluminum, but rather what effect lithium would have on determinations of aluminum and iron that are made by precipitating with ammonium hydroxide in the usual manner, and whether aluminum causes retention of lithium in methods for separating lithium from accompanying elements.

Methods of the type described are about as helpful to the analyst as the method for catching a bird which the old folks used to recommend to children—namely, to sprinkle salt on



its tail. To do that, one obviously must have the bird in hand, and in that case there is no need for the salt. So it is with much of the advice that the analyst receives. Minute directions are given for the salting away of the quarry after it has been separated from its fellows. In this connection, it is interesting to note that when the analyst does happen to encounter a "pure" compound his chief concern is with the impurities rather than the major constituent. In other words, the absolute purity of a pure compound is established by determinations of its impurities.

A contributing factor to the state of affairs that has been outlined was the decision some years back that the analyst was a benighted creature who was working in the dark and sadly in need of enlightenment. And so the structure of analytical chemistry was brought under the spotlight, usually by nonanalysts. Appropriately, the "foundations of analytical chemistry" received first attention. Without question, a study of the foundations of analytical chemistry is eminently fit and proper, provided one finally leaves the cellar. But too many never attempt to reach the upper floors, and so their outlook is restricted and their viewpoint narrow. And they never know the delights of browsing in the attic, and dusting off some of the old antiques that should either be renovated or discarded—heirlooms like the methods for silica and for alumina—not to mention many others.

Of course there is a good reason for confining analytical discussions to determinations which involve one or two variables. A system containing ten to twenty diverse components can hardly be handled on a strictly scientific basis, and any handling of it requires actual experience in analysis. The solubility product of hydrated aluminum oxide can be brought into the picture if ammonium hydroxide is added to a solution of pure aluminum sulfate. But what about precipitations that are made in a solution containing iron, titanium, phosphorus, vanadium, and a dozen other constituents as well? The difference between the two problems is illustrated in Table I. The first case, that of aluminum sulfate, represents chemical analysis as it is not. The second, that of bauxite, represents chemical analysis as it

TABLE I. DETERMINATION OF ALUMINUM

MATERIAL	COMPONENTS										
	Al <sub>2</sub> O <sub>3</sub>	SO <sub>3</sub>	H <sub>2</sub> O								
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·18H <sub>2</sub> O	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	TiO <sub>2</sub>	ZrO <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	V <sub>2</sub> O <sub>5</sub>	Cr <sub>2</sub> O <sub>3</sub>	SiO <sub>2</sub>			
Bauxite	CuO	MnO	CaO	MgO	Na <sub>2</sub> O	K <sub>2</sub> O	SO <sub>3</sub>	H <sub>2</sub> O			

is, and is no exaggeration, for thousands of tons of bauxite are sold, and naturally one of the most important considerations in the sale is the alumina content. If aluminum sulfate is pure enough to warrant precipitation by ammonium hydroxide, followed by filtration, ignition, and weighing of the resultant  $\text{Al}_2\text{O}_3$ , the operations are a waste of time, for one would get the same result much more quickly by igniting the weighed sample and then again weighing. In the case of bauxite, treatment with ammonium hydroxide yields all of the first eight constituents and more or less of the copper. Obviously then, the analysis resolves itself, not to a simple determination of aluminum, but to a complex analysis in which, as a matter of fact, the aluminum ion is never precipitated by itself.

So we have two classes of workers in the analytical field, the determinators and the analysts. The determinators, who are by far the more numerous, may in turn be divided into two general classes: first, the common determinators who follow a method explicitly, without knowledge or concern as to the reactions involved; and second, the educated determinators who can handle systems containing one or perhaps two variables, or who, like determinators of pH, are chiefly interested in group effects and make no effort to go beyond this. The first are the laborers. They need not be chemists, and they require constant supervision. The second are the white-collar workers who exhibit the usual extremes of workers in this classification. The determinator's salvation lies in the development of truly selective methods of analysis, and his final resting place will be a heaven in which he has a shelf containing 92 reagents, one for each element, where No. 13 is the infallible specific for aluminum, No. 26 the sure shot for iron, No. 39 the unfailing relief for yttrium, and so on to uranium.

As for the analyst, he is a comparatively rare bird and is often referred to as a disappearing species, like the old family practitioner who does the best he can, guided by theory and experience, of which the most comforting is experience. It must be admitted that one of the reasons for the gradual disappearance of the analyst is the anomaly that, in spite of the fact that he works entirely with material things, his reward is chiefly spiritual. Who ever heard of a consulting analyst, or of an analyst who patented a new method of analysis or incorporated a company to promote its use? Other reasons for the scarcity of analysts are perhaps the peculiar characteristics, some inherent and some acquired,

that he must possess. He must have the inquisitive habit of a detective, for oftentimes the composition of the material under test is absolutely unknown. He must be an expert manufacturer of pure chemicals, for on this ability the success of his gravimetric determinations depends. He must be an efficient dispenser of liquids of which he knows the exact effect. And, above all, he must serve a long apprenticeship, and keep in constant practice.

So much for the personnel and the general aspects of the project. We come now to the most important details of the job. These are (1) the sample on which the analysis is made, (2) the method of analysis that is used, (3) the accuracy of the result, and (4) the cost of the analysis.

### THE SAMPLE ON WHICH THE ANALYSIS IS MADE

Most analysts are well aware of the difficulties that attend the taking of a truly representative sample of the material that is to be analyzed. Not so well understood are the pitfalls in the way of obtaining the smaller sample on which the analysis is actually made, pitfalls such as (1) differences between the compositions of fine and coarse particles; (2) the fixation of oxygen, water, or carbon dioxide during grinding, sieving, or drying; (3) uncertainties as to the water content after drying and weighing the sample; and (4) changes in the composition of the sample during storage. The extent of errors that may be caused by the first and last are illustrated in Tables II and III.

For examples of errors that may be caused by oxidation, we have but to turn to examples such as the oxide film formed on the particles obtained in machining samples of steel that are to be used in determinations of oxides in steel, the oxidation of sulfur in samples of pyrite that are ground instead of crushed, or the more insidious oxidation of sulfide ores such as sphalerite that takes place during drying or storage. With regard to the fixation of water and carbon dioxide, it is interesting to note that many fired materials and not a few rocks exhibit this property, particularly when in the finely powdered state. For example, samples of a soda-lime glass which had been dried at 105° to 110° C. showed no loss on ignition in the solid state, 0.5 per cent when first ground and sieved, and 1.0 per cent a few years later, even though it had been stored in a can with a fairly close cover. Hydrated materials present special problems, especially if they are to be used as standard samples, for the expulsion of water may be so

TABLE II. DIFFERENCES BETWEEN THE COMPOSITIONS OF PARTICLES IN A SAMPLE

MATERIAL	SIEVE SIZE	COMPOSITION		
		Carbon		
Cast iron	14-20	2.45		
	20-30	2.27		
	30-40	2.04		
Journal bearing		Copper	Lead	Tin
	14-20	70.0	24.4	4.9
	20-30	70.2	24.1	4.9
	30-80	67.6	27.9	4.7
	>80	63.2	31.2	4.4
Ferrotungsten		Tungsten		
	80-100	77.6		
	100-200	80.2		
	200-325	79.5		
	>325	70.4		

TABLE III. CHANGES IN SAMPLES DURING STORAGE

MATERIAL	COMPOSITION WHEN PREPARED	COMPOSITION AFTER STORAGE
	%	%
Ferrovandium	33.6 V	33.1 V
Zinc ore	31.4 Zn	30.4 Zn
Soda-lime glass	74.1 SiO <sub>2</sub>	73.4 SiO <sub>2</sub>

gradual that portions of the same material dried for like periods at 105° to 110° C. retain quite different amounts of water. Finally comes the old question of hygroscopicity, which presents no mean problem at times. Pyrolusite, for example, while not markedly hygroscopic, is sufficiently so to cause entirely erroneous results if two successive samples are taken from the same dried portion. It may be remarked that difficulties in sampling are not confined to commercial materials. Many an analysis of a "mineral" is useless and misleading for lack of care in selecting the sample, and many a determination of atomic weights has been in error because the sample on which it was made was not worthy of the analytical effort that was put on it.

#### METHOD OF ANALYSIS

The second consideration in the chemical analysis of everyday materials is the method of analysis that is used. It is evident that the method should give the result that is desired. It is not so well understood that all methods of chemical determination are in reality based on reactions of groups, and that they can give accurate results for a given member of the group only so long as the other members of the group are absent or their effects can be discounted. Thus, determinations of the chloride ion by precipitation as silver



chloride succeed, provided ions such as bromide, iodide, monovalent thallium, or lead are absent; determinations of iron by reduction with zinc and titration with permanganate leave little to be desired, provided the solution is free from elements such as titanium, chromium, vanadium, or molybdenum; and the electrodeposition of copper is almost perfect unless attempted in the presence of elements such as bismuth, antimony, mercury, silver, gold, or tellurium. Moreover, many do not seem to appreciate that an element in the presence of others may behave quite differently than when it is by itself.

There is no dearth of methods that are entirely satisfactory for the determination of elements when they occur alone. The rub comes in because elements never occur alone, for nature and man both frown on celibacy. Methods of determination must therefore be judged by their "selectiveness." It is in this respect that most methods are weak and that improvements must come, improvements such as are shown in the recently discovered  $\alpha$ -benzoin oxime method, which not only provides a separation of molybdenum from practically all the elements, but also yields a precipitate that can be used for its quantitative determination as well. Think what a boon the discovery of a simple, selective precipitation procedure for the determination of silica would be. Or simple direct methods for the determination of everyday elements such as aluminum, calcium, magnesium, sodium, and potassium, or important commercially used elements like antimony, cobalt, beryllium, boron, and tantalum.

We are also in need of more simple and accurate methods for the determination of substances that are present in very small amount. Most of the present-day methods require entirely too much time, and results are none too satisfactory. It is not uncommon for two determinators to report results which differ by as much as 500 per cent. Such errors are not confined to chemical analysis alone. They apply equally well to estimations made by physical methods, such as spectroscopic, the only difference being that they are not so well known. It might be observed in passing that spectroscopic tests of things as they are are by no means infallible. The term "spectroscopically pure" has a quantitative limitation which is sometimes much more serious than is suspected. In quite a few applications, the spectroscopist is not so sensitive as chemical methods. For example, the chemist can easily discover amounts of molybdenum or anti-

mony in steel, or bismuth in copper that the spectroscopist cannot detect by direct test. The spectroscopist can, however, always excel the chemist by combining spectroscopy with chemical analysis—in other words, by first making separations in which the element that is sought is separated from most of the accompanying elements, and then properly concentrated before the spectroscopic test is attempted. The sensitivity of such tests is bounded only by the analyst's ability to control his blanks.

Because of their lack of selectivity, methods for the chemical analysis of things as they are do not stay put. Therein lies the danger of standard methods of analysis. As soon as the other group members creep in—for example, through changes in manufacture—the method must be changed. We cannot determine tin in steels by the same method that is used for tin in bronzes, not because of the presence of the larger amounts of iron, but because steels are prone to carry group members such as molybdenum and vanadium, whereas bronzes do not—as yet. The most excellent bismuthate method which we use for a plain carbon steel must be discarded when we encounter a steel containing cobalt; the standard procedure for carbon in steel was entirely satisfactory until special steels such as those of the high-sulfur or 18-8 types were made; the evolution method for sulfur in steels fails with those containing titanium, zirconium, or selenium; and the methods for silica and alumina in glasses begin to waver when boron is encountered.

One criticism of present-day researchers in analytical chemistry is that there are too many repairmen as compared with builders. In other words, too many are tinkering on old methods of determination that are scarcely worth the attention, as compared with pioneers who blaze new trails. We have gone about as far as we can go along the old paths. Worthwhile developments must come as a result of new methods of attack, such as the utilization of complex ions, or a systematic study of the reactions of organic compounds.

How far we have advanced in the chemical analysis of things as they are during the past 25 years can be illustrated by the data shown in Table IV.

As for newly proposed methods of analysis, it might be pleaded that the author (1) tell in what respects the method is superior to established methods, including considerations such as time required and accuracy of results; (2) enumerate the substances that prevent its use; (3) outline its desirable



TABLE IV. FIRST RESULTS REPORTED IN COÖPERATIVE ANALYSES OF ARGILLACEOUS LIMESTONE

	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	TiO <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	MnO	CaO	MgO	Na <sub>2</sub> O	K <sub>2</sub> O	S	CO <sub>2</sub>	LOSS ON IGNITION
13.55	3.35	3.53	0.53	0.11	0.08	0.03	41.00	2.06	0.21	0.62	0.18	33.36	34.40
13.87	3.91	1.31	0.036 <sup>a</sup>	0.11	0.11	0.04	41.23 <sup>a</sup>	2.07	0.28	0.62	0.20	33.51	34.45
13.90	3.93	1.57 <sup>a</sup>	0.14 <sup>a</sup>	0.17	0.14 <sup>a</sup>	0.04	41.23	2.10	0.29	0.69 <sup>a</sup>	0.23	33.65 <sup>a</sup>	34.49
13.98	4.08	1.63	0.044	0.18	0.15	0.044	41.24	2.15	0.38	0.74	0.24 <sup>a</sup>	34.12	34.53 <sup>a</sup>
14.02	4.17 <sup>a</sup>	1.65	0.06	0.22	0.16	0.06	41.42	2.17 <sup>a</sup>	0.39	0.74	0.27	...	34.54
14.03	4.23	1.66	0.08	0.25	0.18	0.06	41.58	2.22	0.41	0.76	0.28	...	34.56
14.12 <sup>a</sup>	4.30	1.67	0.12	0.32	0.20	0.12	41.62	2.23	0.43 <sup>a</sup>	0.77	0.29	...	34.60
14.12	4.30	1.73	0.34	0.34	0.44	...	41.64	2.24	0.45	0.82	0.31	...	34.69
14.18	4.68	1.77	...	..	..	...	42.20	2.26	..	..	0.32	...	35.10 <sup>b</sup>
14.30	4.89	1.78	...	..	..	...	42.38	2.40	..	..	...	...	...
AVERAGE DEVIATIONS FROM MOST PROBABLE VALUES													
1930	0.161	0.296	0.22	0.071	0.065	0.021	0.37	0.08	0.08	0.065	0.042	0.225	0.121
1905	0.19	0.25	0.11	0.02	0.02	0.04	0.24	0.13	0.19	0.04	0.02	0.17	...
AVERAGE PERCENTAGE ERRORS IN 1930													
1	7	14 <sup>b</sup>	44	46	59	1	4	19	9	18	0.6	0.4	0.4

<sup>a</sup> Result nearest to the most probable value.<sup>b</sup> 8 if the result 0.53 is omitted.

applications; and (4) tell the whole story in one installment, or at least give a good synopsis of the whole story in the first installment. There is no excuse for passing out information in the "continued in our next" style, which, for example, characterized articles on the use of 8-hydroxyquinoline.

### ACCURACY OF THE RESULT

We have now arrived at the third consideration in the chemical analysis of things as they are—namely, the accuracy of the result obtained. There is a wide misconception, even among analysts themselves, as to the accuracy of the results obtained in analytical work, and many confuse precision with accuracy. It is a comparatively simple matter to establish the precision that can be expected if a certain method of analysis is applied by an analyst or by a group of analysts to a given material. On the other hand, it may be extremely difficult to judge the probable accuracy of a result, for all results are matters of opinion rather than fact, and so the true result is never known. This is one of the most unsatisfactory aspects of chemical analysis. When an analyst scans a bare result, he can never be sure whether the determinator provided for all interfering compounds, hoped that they were absent, or was too ignorant to worry. How difficult the task may be can be illustrated by the data shown in Table V. These represent the results that were reported by thirty different analysts situated here and abroad. It can be seen that 60 per cent of the analysts reported results that were essentially within the average deviation from the general average, and that the average deviation (precision that can be expected if the same analysts determine calcium phosphate in this type of material) is in the neighborhood of 0.33 per cent. But what about the most probable value? Is it the general average, is it within  $\pm 0.33$  of the general average, or is it a value outside of this limit? Anyone who chose the result (77.40) that shows the greatest deviation from the general average would be regarded as taking a very long shot, and yet this is the one which is nearest the correct result. That such difficulties are not confined to everyday analyses is illustrated by the history of the atomic weight of antimony. This was given as  $120.062 \pm 0.0037$  by Clarke in 1920, after most carefully reviewing and weighting the determinations of the atomic weight of antimony that had been made up to that time. He states that "the figure is not quite satisfactory" and "that some more work should be

done." Subsequent analyses bore this out, for 120.2 was adopted in 1921, and the still higher weight 121.77 was chosen in 1925. Note now that this weight is not very far off from the value 121.82 which Clarke obtained by recalculating analyses made by Dumas in 1859, and of which Clarke stated "The values, say all over 121, are almost certainly in error, and ought to be rejected."

TABLE V. ANALYSIS OF PHOSPHATE ROCK

(All analyses on dry basis)

Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> REPORTED BY THIRTY LABORATORIES THROUGHOUT THE WORLD				
%	%	%	%	%
77.40	78.07	78.33	78.61	78.77
77.73	78.16	78.33	78.62	78.79
77.77	78.19	78.40	78.62	78.81
77.84	78.21	78.42	78.63	78.84
77.85	78.24	78.42	78.72	78.86
77.96	78.28	78.60	78.72	79.10

General average, 78.38

Average deviation from general average, 0.33 (14 low and 16 high)

A fair question therefore is: "How can the most probable value be established?" In classical researches, as in determinations of atomic weights, it is chosen by most carefully examining the credentials of the candidate. These must be in minute detail and, in the case of atomic weights, would suffice except for one serious omission, the integrity of the sample, for which no one on earth can vouch. In the analyses of everyday things, the safeguards that attend the acceptance of atomic weights are of course impossible, because cost and time are prohibitive. When arguments arise, industries usually employ an umpire, who often knows less about the game than the disputants, or the results are checked against a standard sample of like composition. How the most probable value is established for standard samples, such as are furnished by the Bureau of Standards, is a long story that can be summarized by the statement that it is based on experience, on the work of others in the field, and usually on determinations made by as many fundamentally different methods as possible.

As for estimating the accuracy of his work, it can be said that the determinator is usually an optimist in thought and expression, while the analyst is a confirmed pessimist. The determinator reports silica in glass as 71.61, if not 71.611. The analyst, who knows that he is doing nicely to insure results in the first decimal place, reports 71.6 and thus is honest with himself and deceives no one as to his powers. The percentage errors that have been made by representative

analysts who have done the best they could in analyses of materials with which they were familiar are shown in Tables VI and VII and emphasize the fact that the ordinary analyst of things as they are is not so much concerned with keeping his errors below one part in one thousand as he is in keeping them below one in one hundred. It should be observed that the accuracy is high in determinations of the high-priced constituents, and that, while the accuracy is not flattering in many of the other determinations, it is still inside the limit at which the data can be used. Moreover, in most cases the accuracy in terms of the material is quite satisfactory. As regards the correlation of data, an error of one point (0.01 per cent) in determinations of carbon in a 20-point (0.20 per cent) carbon steel represents a 5 per cent error, and is yet within the accuracy of ordinary sampling and any use that can be made of the analysis in the ordinary case. With respect to accuracy in terms of the material, we may easily report 0.051 per cent for sulfur in a steel containing 0.050 per cent. This error in terms of the steel is but 1 part in 100,000, or equivalent to measuring a mile to an accuracy of about 0.5 inch, and it has no more significance to the metallurgist at present than the 0.5 inch has to a mile runner, or the time that is recorded. It is also obvious that even larger errors may not be fatal, provided approximately the same error is always made. For example, no harm is done if a glass batch containing 70 per cent of  $\text{SiO}_2$  shows 65 per cent by a method that gives very uniform results and is in daily use in checking its composition. If the actual content is of

TABLE VI. FIRST REPORTS OF ANALYSIS OF CHROME ORE

$\text{Cr}_2\text{O}_3$	$\text{Al}_2\text{O}_3$	$\text{FeO}$	$\text{MgO}$	$\text{SiO}_2$	$\text{TiO}_2$	$\text{CaO}$	$\text{MnO}$
36.62	17.15	14.18	15.95	7.82	0.91	0.32	0.19
36.62	19.37	14.24	15.99	7.86	0.91	0.38	0.22 <sup>a</sup>
36.86	20.28	14.25	16.19	7.99	0.93 <sup>a</sup>	0.44	0.23
36.91	20.55	14.35	16.21	8.05	0.93	0.49	0.35
36.93	20.68	14.36	16.33 <sup>a</sup>	8.21	0.93	0.58	..
36.98 <sup>a</sup>	20.82	14.40 <sup>a</sup>	16.36	8.22	0.98	0.68	..
36.99	20.84	14.49	16.80	8.24	0.99	0.83 <sup>a</sup>	..
37.05	20.84 <sup>a</sup>	14.50	17.49	8.25 <sup>a</sup>	1.06	1.12	..
37.05	20.86	14.56	...	8.28	1.12	1.69	..
37.37	...	14.82	...	8.59	..	..	..
GENERAL AVERAGES							
36.94	20.15	14.42	16.42	8.15	0.97	0.73	0.25
AVERAGE DEVIATIONS FROM GENERAL AVERAGES							
0.15	0.84	0.14	0.37	0.18	0.06	0.33	0.05
AVERAGES OF RESULTS THAT FALL WITHIN AVERAGE DEVIATIONS							
36.97	20.53	14.44	16.25	8.21	0.95	0.60	0.23
RESULTS OUTSIDE AVERAGE DEVIATIONS							
3	1	4	4	4	4	4	2

<sup>a</sup> Result nearest to most probable value.

TABLE VII. AVERAGE PERCENTAGE ERRORS OF DETERMINATIONS OF CONSTITUENTS OF REPRESENTATIVE MATERIALS

CONSTITUENT	MATERIAL	CONSTITUENT %	AVERAGE PERCENTAGE ERROR	CONSTITUENT	MATERIAL	CONSTITUENT %	AVERAGE PERCENTAGE ERROR
Si	Plain steel	0.39	0.86	CaO	Lead barium glass	0.21	8.8
Si	Tungsten steel	0.48	2.6		Burned magnesite	3.36	1.0
Si	Ferrosilicon	75.6	0.2		Argillaceous limestone	41.23	0.28
SiO <sub>2</sub>	Glass	74.1	0.2				
Al <sub>2</sub> O <sub>3</sub>	Glass	0.33	14.2	Mg	Zinc-base die casting alloy	0.10	5.3
	Limestone	4.16	1.8	MgO	Chrome ore	16.30	0.43
	Flint clay	38.77	0.45	MgO	Burned magnesite	85.64	0.06
	Bauxite	55.0	0.08	Na <sub>2</sub> O	Plastic clay	0.27	18.8
					Soda-lime glass	16.65	1.7
Fe <sub>2</sub> O <sub>3</sub>	Soda-lime glass	0.06	9.5	K <sub>2</sub> O	Silica brick	0.29	4.7
Fe	Manganese bronze	1.13	1.7		Lead barium glass	8.38	0.96
Fe <sub>2</sub> O <sub>3</sub>	Plastic clay	2.05	1.4	W	High-speed steel	18.25	0.32
Fe	Hematite	68.60	0.05	Zn	Brass	27.09	0.19
C	Cast iron	2.69	1.4	V	High-speed steel	0.970	0.7
	B. O. H. steel	1.01	0.8		Ferrovanadium	31.15	0.36
S	A. O. H. steel	0.037	3.4	Mo	Nitriding steel	0.163	2.5
SO <sub>3</sub>	Glass	0.41	5.1		Calcium molybdate	35.3	0.16
Mn	Manganese bronze	1.59	1.4	Cu	Cast iron	0.151	5.4
	Ferromanganese	80.67	0.13		Phosphor bronze	78.05	0.03
P	A. O. H. steel	0.037	2.7	Pb	Manganese bronze	0.56	3.1
	Ferrophosphorus	26.2	0.23		Lead-base bearing metal	78.87	0.07
Cr	Cast iron	0.011	0.15	Sn	Lead-base bearing metal	10.91	0.53
	Ferrochromium	67.9	0.12		Phosphor bronze	9.91	0.33
Ni	Nickel steel	3.48	0.34	Sb	Cast bronze	0.16	10.4
	18-8 steel	8.45	0.3		Tin-base bearing metal	7.33	0.81



moment, and the approximate error of a cheap, rapid method is known, the latter is often used and the correction made, as is done in evolution sulfurs on cast iron.

### COST OF CHEMICAL ANALYSES

When we enter the field of chemical analyses of things as they are, we step from the academic into the commercial domain, and so it is proper to discuss costs.

In this field, accuracy and costs usually go hand in hand. So naturally the money that is spent on analyses should not exceed what is necessary to insure the accuracy that is needed. For this reason, in applied analyses we often employ two sets of methods, the cheap routine methods which we use whenever we can, and the expensive umpire or referee methods which we use only when necessary. For example, in determinations of phosphorus in plain carbon steels the rapid alkalimetric method serves entirely satisfactorily for all ordinary purposes, and so the slower and more expensive molybdate-magnesia method is brought out only in cases of serious disputes, or occasional checking of the alkalimetric method.

In any discussion of costs of analyses one should consider the producer as well as the consumer of chemical analyses. If the consumer is at the same time the producer, as in steel works or research laboratories, the situation takes care of itself, for it's all in the family. Consequently, the cost of personnel and of the methods of analysis is, or should be, that which will give the necessary results. In this case, the ratio of analysts to the less expensive determinators depends on the type of work, the former predominating in research laboratories and the latter in plant laboratories. Methods of analysis are also carefully chosen on the basis of speed, dependability, and cost.

Purchasers of chemical analyses, like other buyers, usually get what they pay for, and as a rule are unwilling to pay very much. In purchases of clothing one can buy ready-made or custom-made suits. The former are relatively cheap, because they can be made in quantity from a standard pattern, and are quite satisfactory for the man whose figure follows conventional lines. Similarly, in purchases of chemical analyses we can buy ready-made or custom-made analyses. The former are cheaper, for, like carbon in plain carbon steels, they can be made by a standard operation. They are also



perfectly satisfactory, so long as the composition of the material is not unusual and ordinary accuracy suffices. The moment, however, that you depart from these specifications and enter the custom-made department, costs mount rapidly.

This leaves the producer of analyses—which is no new situation, for he usually gets left. It can be stated at the start that he seldom receives a fair return, except possibly in the case of commercial laboratories which sell ready-made determinations in large volume. Under present conditions there is little profit in custom-made analyses. One reason for this state of affairs is that the analyst sells an intangible commodity, an opinion. Under the circumstances, the purchaser cannot see why he should pay five dollars if someone else is quoting fifty cents. This might not trouble the analyst if the opinion were the result of exclusively mental processes, such as an opinion at law, for then the analyst would not be bothered much by overhead, except when talking through his hat. The difficulty is that the opinion must be based on physical as well as mental effort, and if one discounts the mental, he still has to take care of the physical. It should also be remarked that the purchaser cannot check the correctness of the opinion except by buying other opinions, which is an expensive as well as a poor way to settle an argument.

The intention in this article has been to present a picture of the chemical analysis of things as they are, and not to glorify the analyst on the one hand or to depreciate the state of analytical chemistry on the other. The work of and the demands on the analytical chemist are growing more exacting every day, and we cannot afford to lose the analytical viewpoint.





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## CHAPTER 2

# OPPORTUNITIES FOR ANALYTICAL CHEMISTRY IN SOLID STATE RESEARCH AND ELECTRONICS

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Current practices and problems in analysis in solid state research and development are reviewed. A plea that the analyst assume a broadened responsibility for characterization of materials is made. Examples of solid state problems which were caused by inadequate appreciation and application of analysis are given. In particular, it is pointed out that trace analysis at and below the ppb level, the careful assessment and mapping of physical perfection by x-ray and related techniques and the use of solid-state physical measurements such as conductivity and magnetic resonance will become more important in the future. Mapping techniques and perfection techniques are reviewed in some detail. Recent new methods of stoichiometry assessment are described, and it is suggested that the determination of perfection and defects should become an orderly subdiscipline under the aegis of analytical scientists. The case is made that, insofar as both research and technological progress in solid state and electronics are rate limited by the availability of materials with appropriate properties, it is limited in many cases by our ability to characterize materials. It is concluded that with a modest reorientation of viewpoint and disciplinary content, analytical science could become the core discipline of materials characterization.

Keywords: Analytical chemistry; electronic materials; materials characterization; solid state research; stoichiometry; trace analysis.

## I. Introduction

The electronics industry presently has gross sales of about \$25 billion per year [1]. From a start usually dated from the transistor in the late 1940's, this is a staggering growth. This growth is not complete; competent estimates place sales at about \$32 billion per year by 1974 [1]. Solid state materials, single crystals, ceramics, glasses, polymers and thin



films are the building blocks of the devices from which electronic systems are constructed. Indeed it is axiomatic that the rate limiting process in the progress of present day solid state research and electronic technology is the attainment of materials with desired properties. Several illustrations of technological progress which is materials limited are:

1. Bulk power transmission could be economic with superconducting transmission lines if super conductors with a transition temperature permitting operation at liquid-nitrogen temperature were available. Many researchers even feel that superconductors at or only somewhat above liquid hydrogen would suffice. Present materials come close to being usable in the liquid  $H_2$  range.

2. Bulk information transmission could be economic with laser carriers if a material for a transmission line with a loss at optical frequencies of  $\sim 20$  db/km could be prepared economically.

3. Compact suitcase sized computers might be designed if magnetic materials with small stable magnetic domains whose movement is rapid in modest magnetic fields could be prepared cheaply.

Materials science, the interdisciplinary field which deals with the preparation, characterization, and relationship of properties to bonding and structure, is more and more coming to be the field which assumes the responsibility for the discovery and control of properties of materials for solid state research and electronics. The above examples of materials-limited problems have been chosen partly because they will provide striking and exciting economic benefits if the materials science problems can be solved. The typical daily activities of most materials scientists are usually directed toward more modest ends. However, regardless of the ends, materials scientists' goals are to prepare materials with desirable properties. In order to do this they must attempt an understanding of the connection between chemical composition, physical perfection, and other properties of scientific or technological importance. Thus, the determination of chemical composition and physical perfection is central to progress in materials science and hence to progress in the electronic and related materials industries. The study of materials from this viewpoint has come to be called **materials characterization**.

Frankly, the purpose of this paper is to proselytize for **materials characterization**. Its purpose is to convince you that the new field of characterization needs the analytical chemist and that he will be richly rewarded by adopting its viewpoints. Clearly what is needed is a group of enthu-

siasts for characterization—and in our belief, analytical chemists constitute the logical nucleus for this group.

In the remainder of this paper we examine the problems and successes of characterization, indicate where analytical chemistry presently contributes, and where with slight alteration in viewpoint and disciplinary background it could play an increasingly important role. We ask forgiveness for choosing most of our examples from our own field of electronic single crystals. In addition to it being the field where characterization demands are perhaps most subtle, it is also where techniques of analysis have often been honed to the highest resolution and accuracy.

We also recognize that characterization may not be an ideal word to emblazon upon the new analytical chemist's banner. As W. W. Meinke [2] has pointed out, it does not connote the breadth of the whole of analytical chemistry nor the concern with good sample statistics which is the hallmark of the analytical professional. Nevertheless, we recommend it as a banner for the analytical chemists concerned with materials science, and we particularly recommend that the viewpoints concerning the importance of using a variety of nonchemical techniques be infused in the field. Following some examples of the problems which improper characterization can produce, we will divide our discussion of the field of characterization into the four main areas: Identity of Material; Determination of Major Constituents; Determination of Minor Constituents; Determination of Physical Structure-Perfection and Defects.

Because of space considerations, exhaustive literature references to techniques cannot be given; however, we have tried to provide entry into the literature through a few references on each of the less familiar techniques. It is hoped that at least a few of the techniques will prove new to analytical chemists. Similarly, elaborate documentation of the techniques, materials, and phenomena of solid state electronics cannot be made. Entry references are, however, provided for the less familiar subjects.

We shall begin by defining materials characterization. The most complete study of national needs in the field of materials characterization was made by a special National Academy of Sciences and National Academy of Engineering Committee of the Materials Advisory Board [3] whose definition was:

“Characterization describes those features of the composition and structure (including defects) of a material that are significant for a particular preparation, study of properties or use and suffice for the reproduction of the material.”

Very simply, a material is fully characterized when we know the identity, concentration, and position of all its constituent atoms. Thus, no material can ever be fully characterized; however, many materials have been characterized to a point where relationships between their properties and the location and concentration of their constituent atoms could be made. A whole spectrum of techniques from exotic physical methods to classical analytical chemistry is employed in characterization; but the principal departure from conventionality is in viewpoint.

To understand this viewpoint we will begin by describing what characterization is **not**. Characterization is not the measurement of user oriented properties alone. In electronic materials, user-oriented properties (whether the user is a device designer or a solid state theorist) tend to be measured to death on **uncharacterized** materials. For instance, no matter how important the user may feel these properties to be, either for his theory or for his device, the measurement of conductivity and mobility of a semiconductor single crystal **do not** characterize it.<sup>1</sup> Such measurements in conjunction with other measurements such as careful chemical analysis may be used to determine the nature, concentration and location of those atoms which effect conductivity and mobility. Indeed, physical measurements of semiconductor properties when used together with a sound theoretical understanding of conductivity mechanisms and stringent controls on crystal preparation are among the most sensitive composition and structure sensitive techniques in the characterizer's armory, and when combined with other techniques, are the best means of characterizing semiconductors. Once the number of atoms and their location are known with appropriate accuracy, all of the other properties including conductivity and mobility are determined.

It is interesting to point out that electrical characterization will tell us nothing about electrically inactive impurities. This has been strongly brought home to the semiconductor community by its successive characterization errors involving silicon. Silicon single crystals for some stringent applications are prepared starting with ultrahigh purity trichlorosilane,  $(\text{CH}_3)_3\text{SiCl}_3$ .<sup>2</sup> Many impurities are reduced or even essentially eliminated by careful fractional distillation. The trichlorosilane is then reduced with  $\text{H}_2$  and the resulting Si is further purified and grown into single crystals by zone refining. To prevent contamination from the container during zoning, a crucibleless technique, float zoning, is used. A

<sup>1</sup> Without belaboring the point, we point out that conversely chemical analysis alone does not characterize a semiconductor.

<sup>2</sup> See Laudise [4] for a description of methods of crystal growth; and Kane and Larrabee [5] for a complete review of silicon characterization.

combination of electrical measurements and metallic cation analysis (principally by vacuum spark mass spectroscopy) showed that in Si produced even with the techniques used over a decade ago, metallic impurities were certainly below  $10^{15}$  atoms/cm<sup>3</sup> and probably below  $10^{12}$  atoms/cm<sup>3</sup> (20 ppb - 0.02 ppb atomic). This is indeed impressive and well may make Si among the purest materials man has made. Semiconductor materials people got in the habit of considering that float zone Si had less than  $10^{15}$  atoms/cm<sup>3</sup> total impurities. However, this purity claim completely neglects anions. A rude shock came when careful infrared measurements [6,7] showed as much as  $10^{17}$  atoms/cm<sup>3</sup> of oxygen (2 ppm) in "high purity" Si. The detectability limit for very careful infrared absorption measurements at 9  $\mu$ m (corresponding to an SiO stretch) is about  $2 \times 10^{16}$  atoms/cm<sup>3</sup>, and very carefully prepared Si now has oxygen levels which are below this level.

Can we claim then that our present Si has less than  $2 \times 10^{16}$  impurities? No! Recent work has shown that C contamination from the trichlorosilane (C's distribution constant is unfortunately  $\approx 1$  so that zone refining does not remove it) is probably in the range  $10^{16}$  -  $10^{17}$  atoms/cm<sup>3</sup>. C is usually detected by vacuum spark mass spectroscopy whose detection limit is about 500 ppb. More recently  $\alpha$ -activation analysis shows great promise for C analysis and infrared (IR) measurements at a C-O absorption band have provided increased sensitivity for C in samples of Si deliberately grown with O.

We know less about N<sub>2</sub> but enough to suspect it of being a worse contaminant than C. To round out the picture, fortunately Cl is volatilized during the zone refining and has a low solubility in Si anyway so it is not an important contaminant. However, we must remember that H<sup>+</sup> is a non-metallic cation, very often ignored in semiconductor analysis and thought by some to be as bad an actor in Si as C.

The point for the characterizer to remember is that Si, the lynch pin of solid state research and of the electronic industry, was thought for a long time to have a few ppb impurities—it actually probably has a few ppm impurities. Errors of  $10^3$  are disturbing. No one claims analysis in the ppb range is easy. It can be claimed, however, that it's challenging and important. For instance, if enough O is present in Si (for instance, by growth in an SiO<sub>2</sub> crucible), the quality is so poor that useful devices for many applications cannot be fabricated. Mass demands for Si single crystals at the edge of our ability to control purity and quality will soon be upon us for the silicon single crystal vidicon tube in the PICTUREPHONE®.

Precise measurements on impure materials continue to abound in the literature. When one leaves the Olympian heights of the semiconductor



researchers, impurity and casualness about purity grow apace. I will not give literature citations so as to protect the guilty. Perhaps it is excusable that 1970 papers on the electrical properties of nitrides and carbides do not give information on impurities (even semiquantitative emission spectroscopy) or stoichiometry. Carbides and nitrides are hard to prepare and we may be forgiving. But in spite of the futility of such work, one still finds researchers publishing (and referees accepting) color center studies on halides of unspecified or only vaguely specified purity *e.g.* "purchased from Harshaw in 1968."

Data which W. G. Pfann collected from the literature [3] which are shown in Table 1 reinforce our point. The ratios of resistance at 273 and 4.2 K is often taken as a measure of purity and perfection of metals. Data for 1961 and 1964 are given. Purity and perfection sensitive measurements made on, for instance, Al in 1964 may not agree with those made in 1961 because Al (1961) is not Al (1964). We really ought to know how Al (1961) differs from Al (1964) in order to begin to discuss discrepancies in physical measurements. Most of us usually forget to even recognize that  $\text{Al}_{(1961)} \neq \text{Al}_{(1964)}$ .

Table 1. Resistivity ratios for various metals in 1961 and 1964.

Element	$R_{273}/R_{4.2}$	
	1961	1964
Al	7,000	26,000
Cu	1,700	8,000
Mo	3,300	12,000
Ni	900	2,200
Re	4,000	55,000
W	6,700	80,000

Statements like 5-9's pure are, of course, almost always fraudulent. To know something is 5-9's pure, one must analyze for all of the elements of the periodic table with techniques having resolution of  $\sim 1$  ppm and find that the summation of all the impurities is  $< 10$  ppm. I know of only a few cases where this was actually done, although casual listings of 6-9's (or 4-9's, *etc.*) purity abound. The classic work of Ph. Albert [8] on zone refined Al shows the way. We draw the veil of propriety over other examples and proceed to look at techniques of characterization.



## II. Identity of Material

The grossest question we may pose about a material is "What is it?" For the solids of interest in solid state and electronics, surprisingly, we sometimes answer even this question wrong. The principal reasons for errors of this sort are due to the problem of sampling and homogeneity. The solids of interest in solid state are generally not powders where sampling may be accomplished by variations of the classic methods like coning, tabling, or riffing. The sample is generally a solid body—a glass sample, a single crystal, a solid single piece of ceramic or a polymer. In the most complicated case the analyst must choose a representative number of such samples from either a commercial or research scale production facility, must map individual samples for homogeneity and must choose representative regions of the individual samples for analysis.

### A. MAPPING

Perhaps one of the greatest adjustments of viewpoint required is the willingness to map and the willingness to consider the design and development of mapping techniques a part of the science of analysis. Among the mapping techniques which have been used are:

#### *1. Resistivity Probing [9, 10]*

This technique generally uses small area probes (often four point probes) with special precautions to assure ohmic contact between probe and material. Figure 1 shows a map of a germanium single crystal. Resistivity probing is generally used to reveal impurity or dopant profiles in semiconductors. It can be made quantitative with considerable precision. Spatial resolution of 0.05 in. (determined by the probe size and spacing) is quite easy to achieve. Resistivity variations of  $\pm 20$  percent are quite easy to detect.

When mapping is the object only relative values of resistivity need be compared. However, the real power of resistivity measurements is that in connection with mobility and Hall measurements they allow one to get at the charge carrier **concentration** in a semiconductor. Variations in resistivity can be caused by variations in impurity or dopant concentration effecting carrier concentration, variations in stoichiometry, variations in

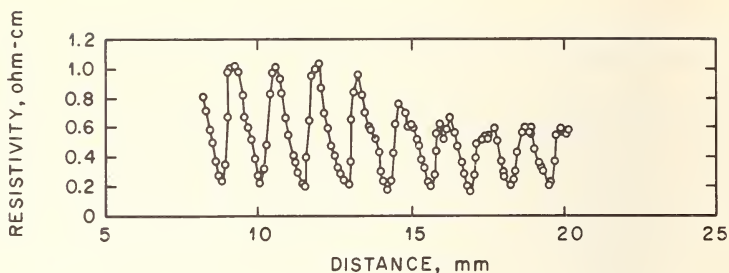


Figure 1. Electrical resistivity map of a Czochralski grown Ge crystal. The distances are measured along the growth axis. Resistivity variations correspond to carrier concentration variations caused by distribution constant fluctuations resulting from temperature and growth rate changes. After Slichter, W.P. and Burton, J. A., in *Transistor Technology*, Bridgers, H. E., Scaff, J. H. and Shive, J. N., Editors, Van Nostrand, Princeton (1958).

impurity content or perfection which affect the mobility of the charge carriers, or variations in impurity content which produce traps which in turn alter effective carrier concentration. In typical semiconductor materials resistivity changes are detectable at carrier concentration ranges down to about  $10^{15}$  -  $10^{16}$  atoms/cm<sup>3</sup>. Trapping centers in the range as low as  $10^{12}$  -  $10^{13}$  atoms/cm<sup>3</sup> can, however, effect carrier lifetime and mobility. Thus lifetime and mobility measurements coupled with trace analysis (see section on determination of minor constituents) are usually essential to make intelligent quantitative use of resistivity measurements.

## 2. Optical Mapping Methods

Optical mapping methods which depend upon index of refraction variations have been used mainly for glasses and crystalline laser and nonlinear optical materials. Figure 2 shows a map of Verneuil grown ruby. Index variation methods reveal impurity, stoichiometry, and strain variations. They are difficult to calibrate perhaps mainly because little effort has been expended, but they can be remarkably sensitive. For instance, it will be shown (in the section on Determination of Major Constituents) that variations of stoichiometry in  $\text{LiNbO}_3$  of less than  $10^{-3}$  cause readily noticeable index of refraction variations. Materials with large stress-optic coefficients will reveal small strains as observable birefringence variations. Readily polarizable atoms or groups lead to large stress-optic coefficients and such groups are required to produce materials with large electro-optic and second harmonic generation coefficients. Thus, the materials of spe-



Figure 2. Striagram of Verneuil-grown ruby—8 inches long and 1/4 inch thick—taken with a point source of white light 35 inches from crystal and with crystal 5 inches from film (no lenses employed). The variations in darkness in the picture correspond to index of refraction gradients caused by  $\text{Cr}^{+3}$  gradients. The curved parallel lines correspond to the growth interface, fern-like patterns are low angle grain boundaries. After Barns, R. L., in *Metallurgy of Advanced Electronic Materials*, Brock, G. E., Editor, Vol. 19, p. 337, Interscience, New York (1963).

cial interest for optical communication studies are also especially susceptible to strain damage and especially susceptible to characterization by observation techniques dependent on index of refraction.

Another important variable which can easily be mapped optically (albeit without much quantitative information being obtained unless considerable effort is expended) is the density of particulate matter. Tyndall scattering of a laser beam is the quickest crude test for particulate matter. Obviously more subtle techniques like electron microscopy or careful angular and wavelength measurements of scattering should be used where a serious evaluation of particulate inclusions is required. Clearly particulate matter whose size is much less than the wavelength of light will not scatter. Indeed many kinds of non-Rayleigh scattering may be missed, but as a quick diagnostic tool, laser light scattering applied as an adjunct of other mapping techniques can often be very useful.

### *3. Etching Studies<sup>3</sup>*

Etching studies reveal a variety of physical imperfections. Etching is especially useful for delineating grain boundaries, dislocations and stained regions due to surface damage in sample preparation. Figure 3 shows an etched specimen of magnesium-manganese ferrite. Usually etching reveals the presence of discrete entities so that calibration is not appropriate.

### *4. Decoration<sup>3</sup>*

Decoration provides a three dimensional map of imperfections. If one diffuses an appropriate impurity into a crystal, it may precipitate at grain boundaries, stained regions and dislocations.

### *5. X-ray Studies<sup>3</sup>*

Techniques such as the Schulz technique provide a good means of mapping low angle grain boundaries. More subtle topographic techniques (*i.e.*, Lang and double crystal topographs) are a means of detecting and mapping strains and dislocations. Precision lattice parameter techniques are a means of mapping the homogeneity of stoichiometry, impurities and of detecting strains.

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<sup>3</sup> Discussed further in the section on Determination of Physical Structure. Laudise [4] gives an elementary discussion of these techniques, lists etches, decoration reagents, and describes the nature and genesis of physical imperfections.



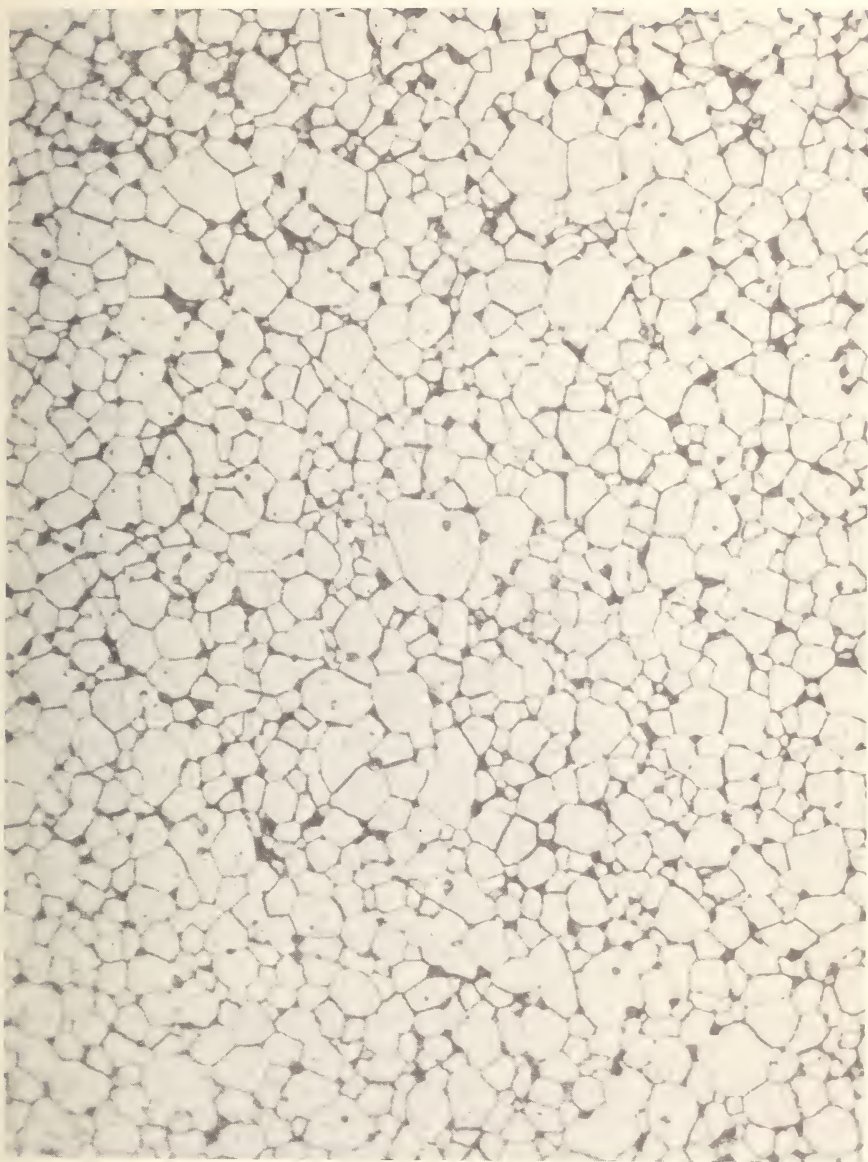


Figure 3. Polycrystalline sample of  $\text{Mg}_{0.675}\text{Mn}_{0.525}\text{Fe}_{1.8}\text{O}_4$  etched 3 minutes at room temperature in  $\text{HF-HNO}_3\text{-H}_2\text{O}$  showing individual crystallite grains (500X). After Monforte, F. R., in Laudise, R. A., Reference [4].



### 6. *Electron Probe Analysis*

The principle, of course, is that of x-ray fluorescence. In essence, a beam of electrons is brought to focus on a spot on the specimen of from 0.1 to 3  $\mu\text{m}$ . The electrons generate the characteristic x-ray spectra to depths of 1 to 3  $\mu\text{m}$  below the surface. Commercial analytical instruments are provided with optics to analyze the emitted x-rays according to wavelength and intensity and a viewing system such as an optical microscope to aid in selecting the exact area to be analyzed. The fact that  $\mu\text{m}$  sized areas can be analyzed and that the probe can be moved systematically, make it an ideal mapping tool. It is especially useful for measurement of particulate inclusions in a matrix, diffusion zones, corrosion layers, and dust particles. Detectability limits are typically something like 100 - 500 ppm. Perhaps a lower limit is 30 ppm.<sup>4</sup> By comparison, the limits in ordinary x-ray fluorescence (albeit on large samples) can be 1 ppm; and in special cases perhaps as low as .01 ppm.

Thus the probe is very useful in what are by many solid state and electronic research criteria relatively dirty samples. For those cases its spatial resolution can be better than 1  $\mu\text{m}$ . It might be pointed out that expressed as a detectability weight limit the probe can find  $10^{-14}$  g in a 10 cubic micrometer volume. From this viewpoint it probably surpasses all other instruments. Also a high ppm detectability limit should not be interpreted too literally or pessimistically. For instance, a local concentration of 100 ppm might correspond to an average concentration of 1 ppb in the whole sample and be undetectable by bulk analysis procedures yet easily found by the probe when it is employed in a mapping mode.

### 7. *Laser Microprobe Analysis*[11]

A very small area of a sample may be easily volatilized with a focused laser pulse. The resulting vapor is excited by a spark or arc and the radiation analyzed by conventional emission spectroscopic techniques. The trace analytical powers of emission spectroscopy are thus combined with the spatial resolution inherent in laser optics. The laser produced vapors might also be analyzed by mass spectroscopic techniques with much increased sensitivity.

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<sup>4</sup> The use of cathodoluminescence can in selected cases improve the sensitivity to 1 ppm. We can expect increasing applications of this technique.

### *8. Autoradiography*

If a material is grown, doped, diffused, or irradiated so as to contain a radioactive species, the uniformity of dispersion of this species can be assessed by exposing photographic film to the sample. Detectability limits and resolution limits will be quite specific to the case at hand. The technique has been used considerably to study diffusion profiles, segregation effects, and the shape of growing interfaces during crystal growth. It has been one of the principal means whereby nonuniformity in dopant distribution in semiconductors has been recognized. Figure 4 is an autoradiograph of germanium.

### *9. X-irradiation*

The production of color centers can sometimes be used to show inhomogeneities in the entities associated with their formation. For instance,  $\text{Al}^{+3} + \text{Na}^+$  are thought to be responsible for the smoky color center of natural quartz. X-irradiation of synthetic quartz produces specimens like that of Figure 5 which reveals inhomogeneities of impurity distribution.

### *10. Electron Micrographs*

Electron micrographs are important in revealing particulate inclusions in materials. Angstrom resolution may be achieved in favorable cases. Identification of the inclusion is, however, much more difficult. The scanning electron microscope has obvious advantages here.<sup>5</sup>

## B. PHASE IDENTIFICATION

The general question, "What phase do I have?" is often given short shrift. X-ray diffraction is the classic method for phase identification and the "Powder Diffraction File"<sup>6</sup> is the phase identifier's bible. It should be pointed out that the file has now been computerized [12] and that Guinier powder patterns are now generally accepted as preferable to Debye-

<sup>5</sup> Field ion microscopy, although more limited in applicability, is actually capable of observing atomic point defects in favorable cases.

<sup>6</sup> Published by the Joint Committee on Powder Diffraction Standards, 1845 Walnut Street, Philadelphia, Pa. 19103.

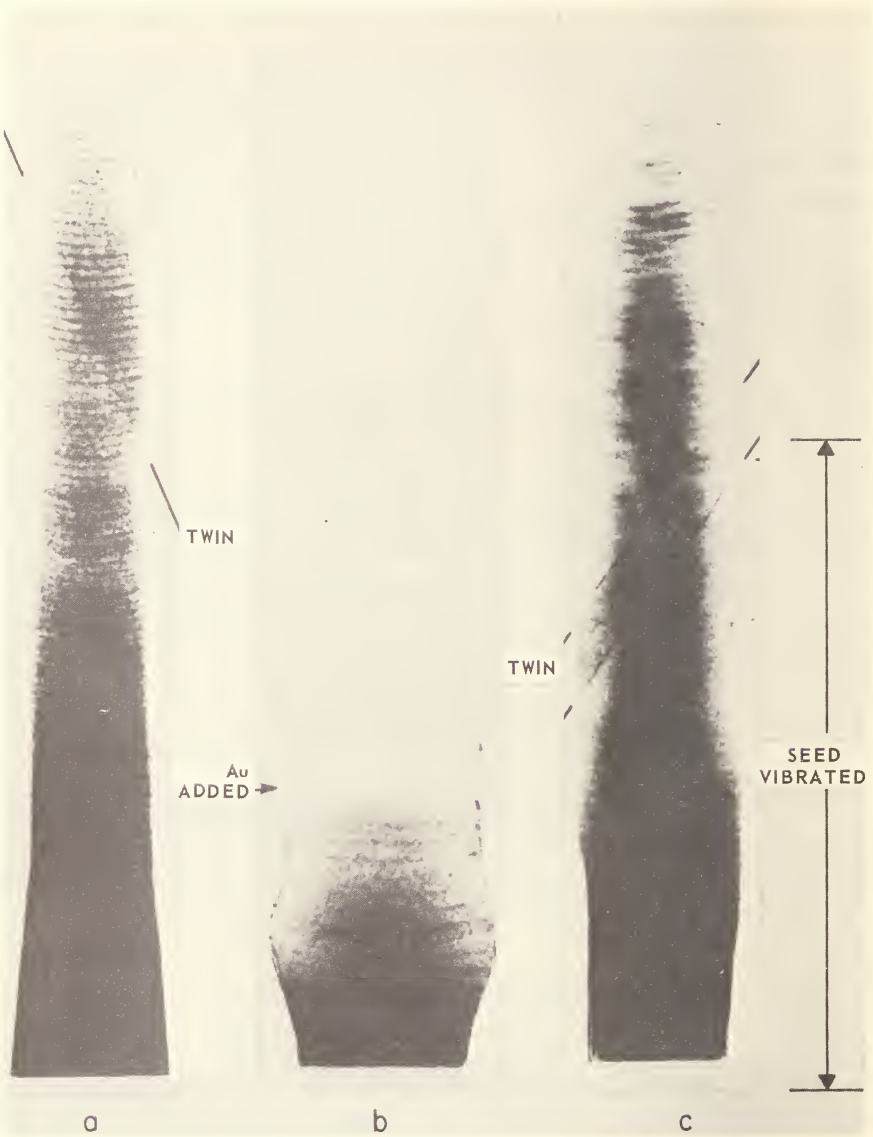


Figure 4. Autoradiogram of  $\text{Au}^*$  doped Czochralski grown germanium crystal—striations correspond to distribution constant fluctuations and demark the growth interface. Courtesy of W. P. Slichter.

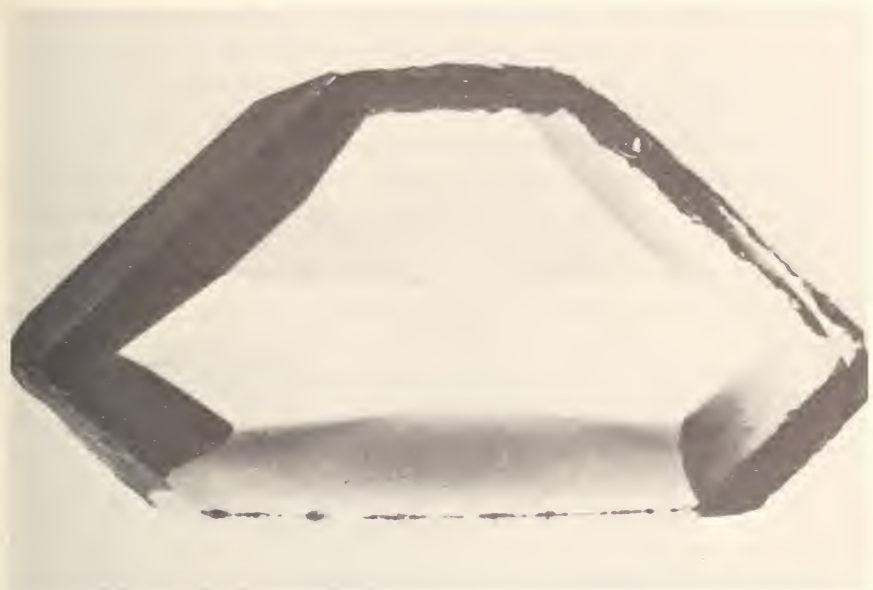


Figure 5. Inhomogeneities in X-irradiated hydrothermal quartz. Sample size is about 3 inches in maximum dimension. The variations in darkness correspond to growth on different faces where the distribution constants for  $\text{Na}^+$  and  $\text{Al}^{+3}$  (which are responsible for the color center) are different. At higher magnification striae corresponding to variation in the distribution constants caused by growth rates and temperature fluctuation are visible. Courtesy of D. L. Wood, after Laudise, Reference [4].

Scherrer-Hull patterns because they provide higher resolution and greater interplanar spacing accuracy. The further automation of diffractometry will be expedited by the recent general availability of systems which directly produces digital data. In ordinary diffraction, diffracting planes in a powder sample diffract monochromatic radiation through various angles. If the x-radiation is white, then at a given diffraction angle radiation of discrete energies (dependent upon the atomic spacing of the diffracting planes) will be detected. Thus, a quantum counter capable of energy discrimination placed at a given fixed diffraction angle can generate digital information related to the spacings in the powder. Li-drifted detectors are appropriate and in use in a recently available system which should give added impetus to automated phase identification [13].

Perhaps the most neglected instrument in the materials analysts' armory is the microscope. If we could make one definite plea, it would be that every instrumental methods course have at least adequate laboratory work devoted to chemical microscopy. A specimen should always be ex-



amed first with a microscope before analysis of almost any sort begins. For a powdered crystalline specimen this will often reveal that what was supposed to be a single phase sample is actually a mixture. Examination in crossed polarizers is helpful in this respect. Crystal habit, index and birefringence often give clues to the identity of the material, simplifying or permitting the omission of later, more time consuming, qualitative analyses. The measurement of interfacial angles and the study of optical interference figures are comparatively simple and rapid procedures which every analyst should have at his command. Standard works on petrography or materials oriented microscopy texts like Bloss [14] should be consulted for the details of technique. Standard compilations [15] of the optical properties of solids which are easily used in identification should be at hand in the analytical laboratory.

### C. PRELIMINARY QUALITATIVE CHEMICAL ASSESSMENT

The classical chemical means of establishing material identity is qualitative analysis. Methods ordinarily thought of as quantitative can often be used qualitatively or semiquantitatively as important aids to identification. Thus, several well known techniques which provide rapid nondestructive analysis eminently suited to identification ought to be mentioned:

#### *1. Emission Spectroscopy*

Perhaps the most generally used method of rapidly assessing a material is emission spectroscopy. Altogether some 70 metallic and metalloid elements can be determined rapidly, generally down to levels of a few ppm. Although ordinarily thought of as a means of determining traces it often turns out that in materials characterization the trace level of interest is considerably below its detection range. However, special procedures have been developed which for certain elements can improve the sensitivity limits considerably. Addink [16,17] gives a good listing of sensitivity limits. Special procedures even allow the determination of anions like C, N, O and the halogens in favorable circumstances at levels as low as 10 ppm. Nevertheless, in modern characterization the real forte of emission spectroscopy is rapid-survey-assessment, that is, establishing identity and establishing what impurities in general are present above the ppm level. Its other strength is for the careful quantitative analysis of



traces in the ppm-percent range. Obviously chemical preconcentration is a general way of improving the sensitivity of spectroscopy and most other instrumental methods. The limits to which preconcentration can be pushed are set by trace impurity introduction and losses of constituents whose determination is desired during the preconcentration steps and will, of course, be unique for each element and preconcentration procedure.

## *2. X-ray Fluorescence*

Ordinarily thought of as a tool for the quantitative analysis of major and minor constituents, x-ray fluorescence spectrometry can also be very useful in qualitative identifications. Its precision is often competitive with tedious quantitative wet analysis and even more often superior to wet qualitative analysis. The fact that it is nondestructive is advantageous. Its sensitivity is from  $\sim 5$ -20 ppm for elements with atomic numbers above Ti to  $\sim 50$ -200 ppm for elements with atomic numbers near C. More effort on the effect of valence, coordination, electronegativity and structure on the shape, position and intensity of long wavelength x-ray spectral lines and absorption edges could pay handsome dividends in turning x-ray fluorescence into a characterization tool of greater power and discernment.

## *3. Other Instrumental Techniques*

Most of the techniques ordinarily used for the determination of major phases and many of those used for trace analysis can be used in a survey mode for assessing identity. Some of these methods are discussed in appropriate sections below, but for completeness flame emission and atomic absorption spectroscopy, spectrophotometry and polarography, should be mentioned here as often having very useful roles to play in preliminary surveys of identity. The possibilities of automating wet inorganic qualitative analysis in a manner which has proven so successful to clinical analysis should be considered.

As a part of materials characterization the determination of phase diagrams and the use of the phase rule viewpoint in considering homogeneity ranges, stoichiometry problems, and defect formation should be emphasized. The recent development of rapid, reliable, comparatively in-

expensive differential thermal analysis (DTA), thermalgravimetric analysis (TGA), and calorimetric apparatus should do much to expedite this approach. In particular, the determination of polymorphic phase transitions and exsolution regions is important. Because a physicist knows the identity of his material at room temperature, he has **no** reason to assume (without characterization studies) that it is similar, for instance, at liquid helium temperatures. High and low temperature x-ray studies are thus an important adjunct of characterization. Dilatometric studies, dielectric studies and optical studies conducted as a function of temperature are also often needed to establish whether a material undergoes a phase change. To establish uniquely the order of a phase change, the most definite measurements, of course, are heat capacity determinations. In particular, we would recommend the excellent series edited by Alper [18] as an introduction to the phase equilibria viewpoint and the techniques of diagram determination from the materials science-characterization viewpoint.

Obviously one analyst cannot be a high temperature calorimeterist, a low temperature x-ray diffractionist, a dielectric studies expert and seven other kinds of esoteric specialist. However, in addition to fulfilling the role of analytical chemist the competent characterization group should know when the services of some of these experts are needed, and understand their strengths and limitations. Figure 9, the phase diagram of a part of the  $\text{Li}_2\text{O} - \text{Nb}_2\text{O}_5$  system which will be discussed below, well illustrates the sort of characterization insight the phase equilibria viewpoint can produce.

### III. Determination of Major Constituents

Analytical chemistry has its center of gravity in this area. Most analytical techniques are more than good enough to establish an empirical formula with great confidence for a Daltonide compound where  $\pm 1$  percent is often acceptable. The principal problem in determining major constituents in electronic materials analysis is in obtaining precision enough to determine stoichiometry in Bertholide compounds. Thus, stoichiometry determinations will be the main subject discussed in this section. It is probably true that conventional volumetric and gravimetric analyses are sufficient, if only we would apply ourselves to determine elements and hence stoichiometry within  $\sim \pm 0.1$  percent. After all, T. W. Richards Nobel winning atomic weight determinations based on classic analytical methods showed over sixty years ago that careful conventional

techniques, principally gravimetry and nephelometry, permit the determination of chemical atomic weights to five significant figures. It might be assumed that similar techniques carefully applied today could permit analysis to similar precision. If anyone is still interested in the lengths to which careful technique can push conventional methods, they might read Richards' papers [19].

Kane and Larrabee [20] review the difficulties of determining stoichiometry in compound semiconductors. Two examples taken from recent work in Bell Laboratories will perhaps show the importance of stoichiometry in electronics research and development and illustrate some novel methods of determining it.

The importance of nonlinear optical materials for solid state research and development has been reviewed elsewhere (Laudise, Carruthers and Jackson, 1970) [21]. Suffice to say that optical communications hinges to a considerable degree on obtaining high quality modulator and parametric oscillator crystals. Modulator crystals are needed to impress the desired signal onto optical carriers and parametric oscillator crystals are required to generate a variety of carrier frequencies. Modulator crystals must exhibit a large electro-optic effect, that is, a large change in index of refraction with applied electric field. Parametric oscillators require crystals with a large coefficient of harmonic generation, that is, crystals which efficiently convert coherent laser light of a given frequency to a doubled frequency.<sup>7</sup> For both effects the polarization must depend in a nonlinear fashion upon the field—hence, the phenomena are called nonlinear optical effects. Lithium niobate was one of the first practically useful nonlinear materials discovered and it continues to be of high importance as a parametric material. The problems of stoichiometry observed in this material and the methods of observation and correction are probably generally typical of most of the nonlinear niobates and tantalates [22].

For harmonic generation it is necessary that the index for the generating wave equal the index for the generated (doubled) wave throughout the crystal. The dispersion in index with wavelength is compensated for by the use of materials whose birefringence exceeds their dispersion. In such materials an appropriate choice of direction and temperature leads to index and phase matching for generating wave and harmonic. It is thus important that the index and birefringence be extremely uniform throughout the device crystal (typically several cm in length).

Crystals are grown by the Czochralski technique (pulling from the melt). Figure 6 shows the dependence of indices ( $n_o - n_e$  = birefringence)

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<sup>7</sup> The frequency is not always double the generating frequency, but for tutorial purposes, we will discuss this simplest case.

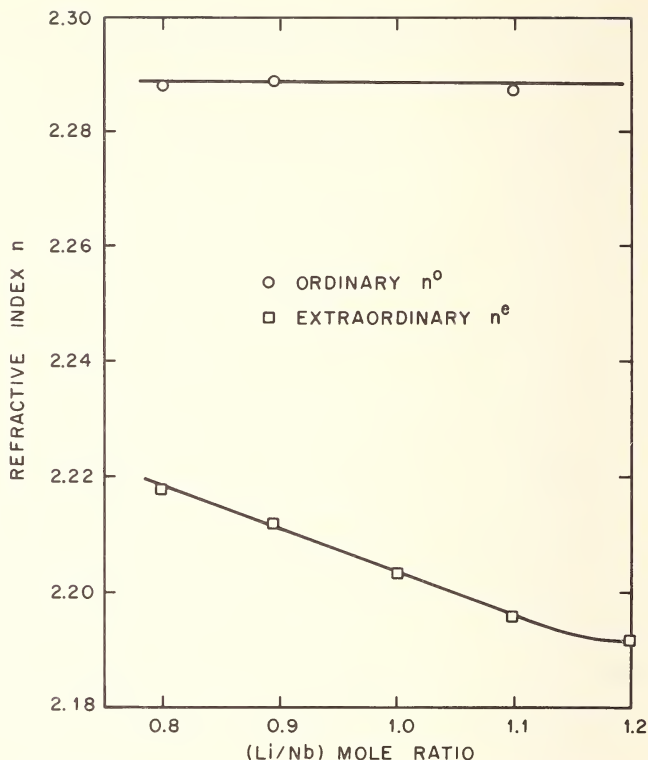


Figure 6. Refractive index at 6,328 Å of  $\text{LiNbO}_3$  as a function of Li/Nb mole ratio in the melt from which the crystal was grown. After Bergman, J. G., Ashkin, A., Ballman, A. A., Dziedzic, J. M., Levistein, H. J., and Smith, R. G., *Appl. Phys. Letters* 12, 92 (1968); see also Carruthers, J. R., Peterson, G. E., and Bridenbaugh, P. M., *J. Appl. Phys.* (in press) for data on distribution constant and Bridenbaugh, P. M., *J. Cryst. Gr.* (in press) for most recent data on growth.

upon the  $\text{Li}_2\text{O} - \text{Nb}_2\text{O}_5$  ratio in the melt from which the single crystals are grown. From Figure 6 and the requirement that variations in birefringence be less than  $\sim 10^{-5}$ , the requirement that melt stoichiometry not vary more than  $\sim \pm 0.2$  percent arises. If the phase diagram  $\text{Nb}_2\text{O}_5 - \text{Li}_2\text{O}$  is such that for the compound  $\text{LiNbO}_3$  the maximum melting point does not occur at  $\text{Li}_2\text{O}/\text{Nb}_2\text{O}_5 = 1.00$  (i.e.,  $\text{LiNbO}_3$  melts "non-congruently"), then we can expect that  $\text{Li}_2\text{O}/\text{Nb}_2\text{O}_5$  and  $\Delta n$  along a grown crystal will look like Figure 7. Indeed Figure 7 is a superb example of careful mapping. The linear variation is due to the fact that segregation takes place during freezing, i.e., if for instance, the composition of the melt is such that the



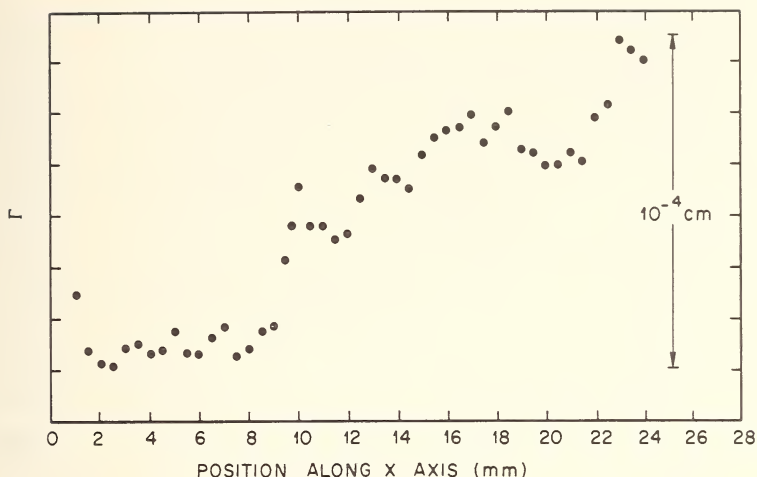


Figure 7. Birefringence as a function of length in a Czochralski grown  $\text{LiNbO}_3$  crystal—the melt composition was  $\text{Li/Nb} = 1$ . The mapping wavelength is  $0.63 \mu\text{m}$ . The ordinate is the net phase length introduced by a compensator and is proportional to the birefringence. After Nash, F. R., Boyd, G. D., Sargent, M. III, and Bridenbaugh, P. M., *J. Appl. Phys.* **41**, 2564 (1970).

freezing crystal is richer in  $\text{Li}_2\text{O}$  than its melt,  $\text{Li}_2\text{O}$  will be rejected upon freezing the melt and the crystal will thus grow richer in  $\text{Li}_2\text{O}$  as it is pulled. Another way of stating the situation is to say that the effective distribution constant  $k_{\text{eff}} = (\text{Li}_2\text{O concentration in the crystal}) / (\text{Li}_2\text{O concentration in the melt}) \neq 1$ . The random fluctuations superimposed upon the linear variations are due to growth rate and temperature changes altering  $k_{\text{eff}}$ . To further understand and control the phenomena one needs extremely accurate characterization and mapping of stoichiometry variations in the crystal. Fortunately, G. E. Peterson [23] showed recently that broad linewidth  $^{93}\text{Nb}$  nuclear magnetic resonance is extremely sensitive to stoichiometry in  $\text{LiNbO}_3$ . Figure 8 [23] shows that the linewidth can be used to distinguish  $\text{Li}_2\text{O}/\text{Nb}_2\text{O}_5$  to within  $\pm 0.2$  percent. Figure 8 was made with powder samples of  $\text{LiNbO}_3$  which are prepared by carefully sintering, grinding, and re-sintering of  $\text{Li}_2\text{CO}_3$  and  $\text{Nb}_2\text{O}_5$  with several re-firings and x-ray Guinier pictures used as tests for complete reaction so as to insure sample integrity. Such procedures combined with very careful weighing of pure starting materials enabled the final composition of the  $\text{LiNbO}_3$  powders and hence the accuracy of Figure 8 to be established to  $\pm 0.2$  percent. Single crystals give similar resonance signals so that Figure 8 can be used to determine the composition of



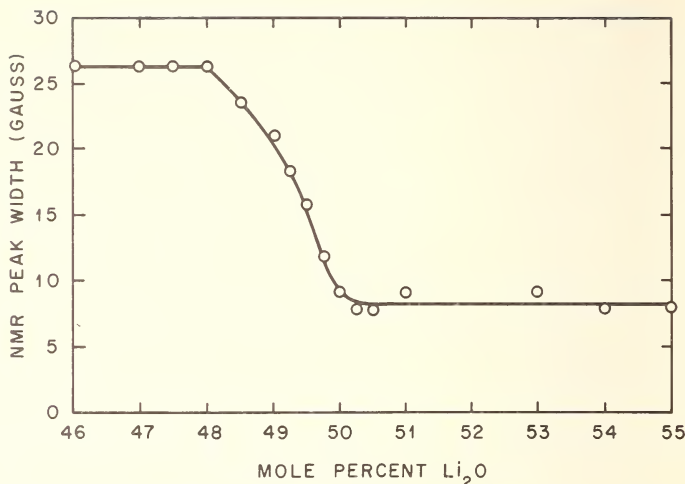


Figure 8. Variation of half-width of low field derivative  $^{93}\text{Nb}$  NMR peak as a function of composition for  $\text{LiNbO}_3$  ceramic specimens. After Peterson, G. E. and Carruthers, J. R., *Sol. St. Chem.* 1, 98 (1969).

crystals grown from melts of various compositions. An additional powerful technique for deducing composition in ranges where nuclear magnetic resonance (NMR) sensitivity is unsatisfactory is the careful comparison of ferroelectric Curie temperatures between ceramic samples of known composition and unknown composition melt grown specimens [24].

These methods combined with conventional techniques enabled the careful determination of the phase diagram which is shown in Figure 9 [25]. Birefringence mapping (Fig. 7) plus techniques based upon the careful determination of the temperature dependence of second harmonic generation [26] were used together with resonance linewidth measurements of sections of grown crystals to determine the stoichiometry variation along a grown crystal. These techniques clearly demonstrated that as the phase diagram would predict crystals grown at any stoichiometry except 48.6 percent  $\text{Li}_2\text{O}$  (the maximum in the melting point, where the liquid and solid compositions are equal) showed extreme linear stoichiometry and birefringence variations from end to end and large random fluctuations due to growth rate and temperature fluctuations. Such crystals were not usable for devices. Crystals grown at 48.6 percent  $\text{Li}_2\text{O}$  where the liquid and solid compositions were identical ( $k_{\text{eff}} = 1.00$ ) were extremely homogeneous and gave good figures of merit in devices. Thus stoichiometry characterization and control proved an important key to the problem of securing homogeneous crystals for optical applications.

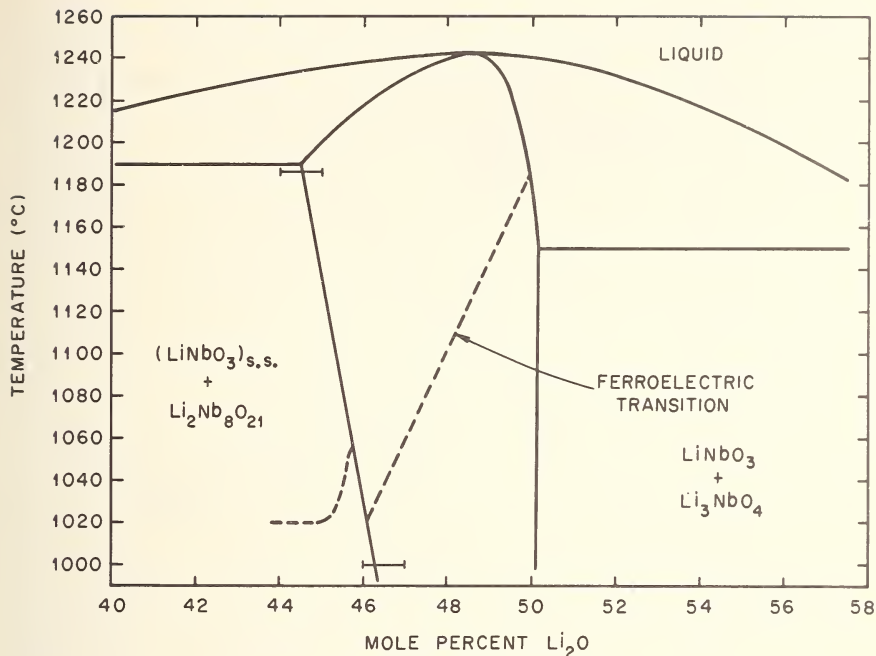


Figure 9. Part of the  $\text{Li}_2\text{O}-\text{Nb}_2\text{O}_5$  diagram. After Carruthers, J. R., Peterson, G. E., Grasso, M., and Bridenbaugh, P. M. (to be published).

Figure 10 shows the results of a similar study of linewidth in gallium phosphide single crystals [23]. Gallium phosphide is pulled from a melt encapsulated under liquid  $\text{B}_2\text{O}_3$  at high pressure to prevent P evaporation, and is an important electroluminescent device material whose electroluminescent efficiency can vary by large factors for reasons considered by most researchers to be obscure. Figure 10 strongly suggests a "stoichiometry problem" in GaP with growth being carried out presently away from the congruent maximum in the Ga-P diagram. Confirmatory experiments are underway.

Whenever nuclei of appropriate spin are present, we may expect linewidth measurements to become an increasingly powerful tool in stoichiometry studies.

Less tractable cases will still require techniques such as the comparison of x-ray and hydrostatic density. Even in cases where nonstoichiometry is well established, density comparisons can shed light on the mechanism of charge compensation in off-stoichiometric materials. Typical of this is

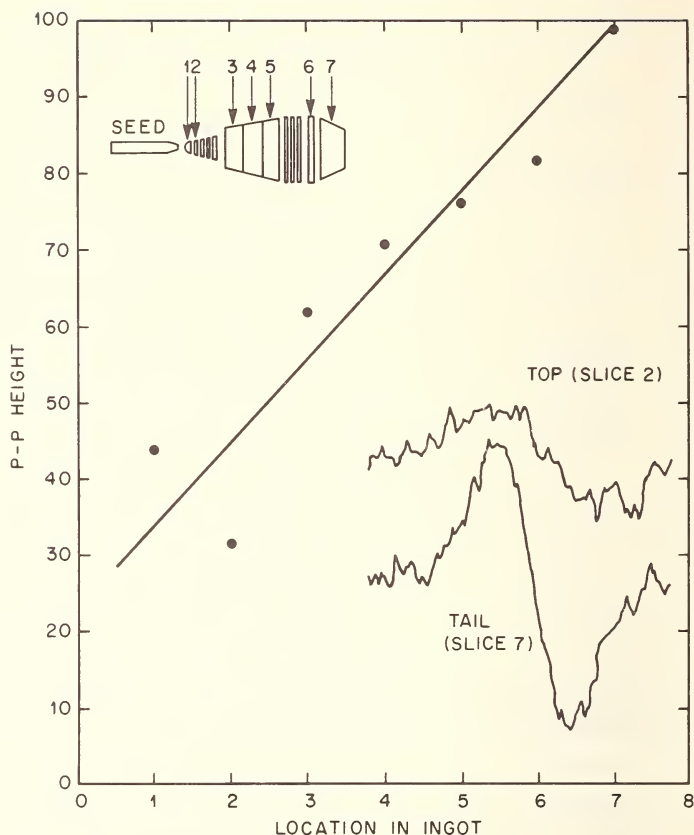
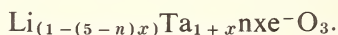


Figure 10. Peak-to-peak NMR derivative height vs ingot location in pulled GaP normalized so that P-P height = 100 = starting material. After Peterson, G. E., Carnevale, A. and Verleur, H., *Mat. Res. Bull.* **6**, 51 (1970).

Barns' and Carruthers' study [28] of  $\text{LiTaO}_3$ . The generalized defect model formula which resulted from this study is



The existence region was found to be 46.0 to 50.4 mole percent  $\text{Li}_2\text{O}$  at room temperature. Charge compensation by oxygen vacancies was deemed implausible on the basis of optical spectroscopy studies. Since the pycnometric density was found to be larger than the x-ray density, it was reasonable to assume that the excess tantalum atoms are compensated for

by lithium vacancies and by excess electrons. Depending upon the stoichiometry the value of  $n$  varies between 0 and 2; the most probable values suggesting that the formula is  $\text{Li}_{1-4x}\text{Ta}_{1+x}\text{xe}^-\text{O}_3$ . Presently scatter in  $n$  values when data is fitted to the densities causes considerable concern. This is somewhat mitigated by the fact that  $n$  values calculated using only the Li/Nb ratio are in agreement [29]. Improvements in pycnometric density and Li/Nb ratio determinations are required, especially if one wishes to search for oxygen lattice defects.

Coulometry is applicable both to major constituent and trace analysis. It can be especially precise for the determination of major constituents. Reliability at the 0.002 percent level can be obtained in favorable cases. Thus, it could find an important role in stoichiometry determinations. Marinenko [30] at the National Bureau of Standards has recently shown that constant current coulometric titrations with amperometric end point determination can determine stoichiometry with great precision. One important advantage is that since the technique is essentially titration with electrons, its accuracy can be independent of calibration of standards. Provided the reaction actually going on is the reaction calculated and provided no losses occur in preparing the material for reaction, the precision obtained may in the limit be set by the precision to which we know the value of the Faraday.

In nonstoichiometry above 0.1 percent, volumetric methods are conventionally employed. For instance, Bachelder and Sparrow [31] fused  $\text{InSb}$  with  $\text{Na}_2\text{CO}_3 + \text{S}$  and then dissolved in dilute  $\text{HCl}$ . After oxidation of any unreacted  $\text{S}$  with  $\text{KClO}_4$ , the antimony was titrated iodimetrically.

Polarography is applicable to trace, minor, and sometimes even major, constituents in solution. In principle any element subject to a redox reaction can be determined. The behavior of some 80 elements has been discussed. Sensitivities of from 0.1 to 0.001 ppm can be obtained. The use of this technique for trace analysis and minor constituent analysis is well known. Its precision near the detectability limit is usually reported as  $\sim 20$  percent generally with precisions near 1 percent in favorable concentration ranges. Differential techniques have pushed precision to 0.02 - 0.05 percent in some cases. Serious application of the technique for stoichiometry determination would now be appropriate.

Perhaps the most sensitive stoichiometry dependent property is lattice parameter. Calibration here is a problem. In the simpler case where one considers substitutional impurities on a lattice, estimates of accuracy are possible. If we assume Vegard's law is obeyed, when two end members have lattice parameters differing by 10 percent, then given a standard deviation in the lattice spacing measurement of 1 ppm the concentration

of one in the other can be determined to 0.001 percent. Barns has shown that these numbers can be realized in practice [32].

In addition to stoichiometry determination, the other main problem of major constituent analysis in electronic materials is the determination of oxidation state.

The precise determination of oxidation state of an element in a compound is a recurrent problem. To a first approximation some difficulties of oxidation state determination are semantic rather than analytical. They arise because of confusion between formal charge and **real** charge. The chemist or materials scientist has every right to ask the analyst to help him determine formal charge. The determination of **real** charge is much more subtle and has only recently begun to be capable of measurement. Once exact stoichiometry has been determined, it is conventional to round off to the nearest whole numbers and to assign the formal charge (*i.e.*, valence) so as to preserve electrical neutrality. This presents difficulties requiring analytical assistance when more than one multivalent element is present in the same compound or where several valency assignments are possible for a given element in the compound, *e.g.*, iron in Prussian blue.

Classical wet chemical and electrochemical methods are applicable, if one can assure himself that dissolving doesn't alter valence. A precision of perhaps 0.01 percent might be expected in fortunate cases. For appropriate nuclei (Fe, Sn, I, Au, and some rare earths) Mössbauer spectroscopy is applicable with a precision of 0.1 percent and no worries about the need of dissolving. The detectability limit is somewhere around the fraction of 1 percent level in favorable cases. Mössbauer spectroscopy has shown that the oxidation states of the Fe in Prussian blue are  $\text{Fe}_4^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6]_3$  [33]. Visual color observation and absorption spectroscopy is also sometimes useful in establishing oxidation state.

At a higher level of subtlety we may ask what is the true charge on a particular element, taking into account its actual environment, degree of covalency of its bond, *etc.* nuclear quadrupole resonance (NQR) spectroscopy can, in some instances, provide interesting insights into this question. Figure 11 shows the results of some NQR studies in which the actual charges were calculated. These studies are especially interesting because they provide one of the first direct measurements of one of the chemist's favorite concepts — degree of covalency.



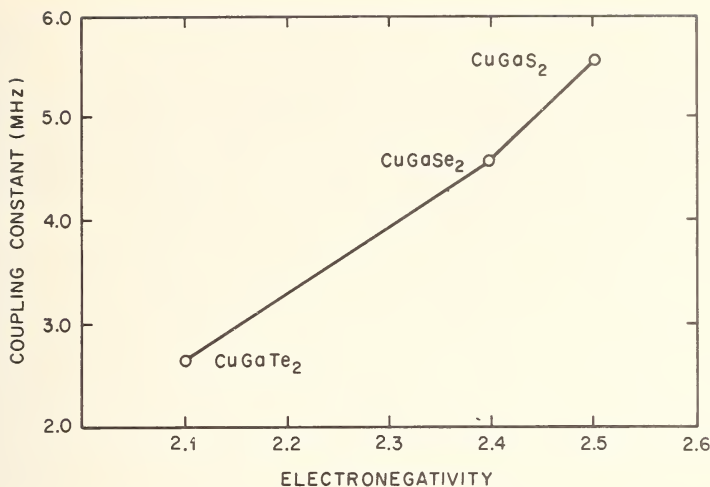


Figure 11. Nuclear quadrupole coupling constant of  $^{63}\text{Cu}$  in chalcopyrites plotted against the electronegativity of the chalcogen. After Peterson, G. E., in *Magnetic Resonance*, Coogan, C. K., *et al.*, editors, Plenum Press, New York (1970).

#### IV. Determination of Minor Constituents

The analysis of impurity levels in the range of from 0.1 percent to 1 ppb received great impetus from the necessity of assaying and controlling trace elements in semiconductors. There are three mainstays of trace analysis:

##### A. EMISSION SPECTROSCOPY

This is probably the most widely used tool for assessing the quality of pure materials. It has found its full glory in the metals industry, especially in basic oxygen steel making where automatic spectrographic analysis coupled with computer process control provides direct feedback of the results of analysis into the process. The principal reason why emission spectroscopy has found such wide acceptance in metals processing (other than economies of scale which enable the results of one series of analyses to be used to control the properties of many tons of steel) is that the concentration range of interest from  $\sim 100$  ppm to 1 percent is generally in the range of good sensitivity.

For the technique to be generally useful in the impurity range of interest in semiconductors either preconcentration or refinement of the source is

generally required. Preconcentration techniques have been used for electronic materials spectroscopy, especially for Ge, Si, and GaAs. However, preconcentration procedures all suffer from inaccuracies due to the necessity of introducing additional reagents (which are contaminant sources) in the chemical preconcentration steps and due to the unavoidable losses which occur during those steps. Thus, direct methods are almost always to be preferred if appropriate sensitivity can be achieved.

The principal problem for Si and Ge is that oxide bands obscure the spectrum of most impurities. The conventional expedients are to strike the arc in argon or nitrogen or to use the so-called split-burn technique. Since impurities are selectively volatilized in the arc, the background can be reduced with respect to any one impurity by moving the plate during the burn. Because the oxide problem is less, the better sensitivity is obtained in compound semiconductors like GaAs. Generally the important anions, especially oxygen, sulfur, and the halogens cannot be determined with reasonable sensitivities by emission spectroscopy.

## B. FLAME EMISSION AND ATOMIC ABSORPTION SPECTROSCOPY

These two methods are complementary in that often elements with poor detection limits for one technique have good limits for the other. In favorable cases 0.01 ppm can be detected in solution; a few ppm is comparatively routine. Alkalis and alkaline earths have the best detection limits, while metaloids and metals have poor detection limits. Matrix effects, dilution effects by dissolving, and contaminant interferences are problems.

## C. VACUUM SPARK MASS SPECTROMETRY

Mass spectroscopy is of broader application and of higher sensitivity than emission spectroscopy. Modifications of Hannay and Ahearn's spectrometer [35] have been commercially available for over ten years and are now the work horses of trace analysis in most solid state laboratories. The general rule is that sensitivity is  $10^2$  better than emission spectroscopy (Cu is an important exception, emission spectroscopy is more sensitive). About 3 ppb atomic is possible and the Picker-Nuclear bulletins are recommended as an especially comprehensive listing of sensitivities for various elements [36].

## D. ACTIVATION ANALYSIS

Emission and mass spectroscopy are probably most valuable because they are comparatively rapid survey methods. Actually their sensitivity does not reach to the limits of the levels where impurities can cause electrical effects. For instance, perhaps the best that can be expected from mass spectroscopy is a limit of 2 ppb atomic which corresponds to  $\sim 10^{14}$  atoms/cm<sup>3</sup> on Si. This is not an unusual doping range; dopings to 1/5, and even occasionally 1/10 this level, are not unknown. Undesired impurities which act as traps can have effects much below this level. When the proper conditions are met, activation analysis can reach to these levels. Generally only the highest thermal neutron fluxes such as are obtainable only in large nuclear reactors give sensitivities of interest for the impurity levels found in semiconductors.<sup>8</sup> Two classes of impurities are amenable:

1. Those with high enough neutron cross sections and long enough half lives so that the induced activity can be dealt with (at fairly low impurity concentrations) at sites removed from the reactor. That is, half lives greater than  $\sim 24$  h still permit reasonable induced activities.

2. Those whose cross sections are small but have short half lives less than  $\sim 24$  h so that reasonable activities, and hence impurity concentrations, can be dealt with if measurements are made quickly following irradiation.

Obviously when half life is long and cross section is small, the element may not be detected. Interferences can be dealt with by chemical separation, by energy and particle dependent radiation detection, and by distinguishing materials of different half lives. The ideal cases where no contamination or losses will occur are those where no chemical processing is necessary. Some work on the analysis of anions by activation analysis has been published, but much remains to be done in this area. When specific activity requirements are met, an important limitation in reproducibility is often sample homogeneity. Perhaps the most important advantages of activation analysis are that often chemical processing can be avoided and usually the problem of standards is obviated because direct calculation of concentrations from absolute activity is possible. One cannot claim to be seriously involved in trace analysis at the levels of interest for electronic materials unless he has activation analysis capabilities.

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<sup>8</sup> Ion activation analysis (*e.g.*, He<sup>3</sup> for O in Si and GaP) and  $\alpha$ -activation analysis (*e.g.*, for C in Si) are important activation techniques, enabling the determination of materials where interferences or neutron cross section-half life considerations limit the sensitivity of neutron activation.

### E. ISOTOPE DILUTION

Isotope dilution is useful when cross section-life time constraints prevent the attainment of the desired sensitivity by activation analysis. The method is advantageous in that quantitative separation methods need not be available for the system under study. All that need be measured is the specific activity of the isotope added, its weight, and the specific activity of an aliquot taken from the sample after one has assured that the added isotope is uniformly dispersed. To assure uniform dispersion it is helpful if the isotope and the sample can be dissolved. Accuracy comparable to activation analysis is possible. The technique is limited by the availability of isotopes of appropriate activity of the elements desired. It clearly should be used much more often for trace analysis.

### F. COPREX TECHNIQUE

C. L. Luke and his coworkers [37] have used preconcentration in combination with x-ray fluorescence to achieve sensitivity levels in the ppb-ppm range. The unknown is dissolved and the desired element or elements are precipitated with an appropriate group reagent and isolated by filtration onto, for instance, a Milipore filter. Conventional x-ray fluorescence of the filter gives very good sensitivities and contamination and losses in the preconcentration steps can be kept to a minimum. The technique can rival radiochemical techniques for sensitivity and obviously has advantages of convenience.

### G. OTHER METHODS

Nuclear magnetic resonance studies have been applied to a variety of trace analysis problems. Sensitivity varies with the type of nucleus present but is generally in the  $10^{19}$  nuclei/cm<sup>3</sup> or 0.1 atomic percent range. However, double resonance schemes can push sensitivity to the  $10^{16}$  range. Electron spin resonance is useful for detecting paramagnetic atoms and electrons. The optimum cases are in the range  $10^{16}$  spins/cm<sup>3</sup>, which can make it fantastically sensitive for free radicals, color centers, and transition metals. The usefulness of resonance as a general analytical tool depends to a large degree upon inventive modifications of existing techniques and the willingness of resonance researchers to undertake characterization.



Vacuum fusion is, of course, useful for determining minor constituents when they are gaseous. It can also be a very sensitive method for analyzing for traces. Recently more rapid apparatus [38] allowing 60+ samples/day to be analyzed makes the technique considerably more attractive. Precision and accuracy depend to an unusual degree upon the calibration situation.

Other methods of trace analysis which have been applied with some degree of success to semiconductor materials include fluorometric analysis, polarographic analysis, and infrared absorptometry. IR absorption has proven especially useful in characterizing oxygen in Si and was discussed in the Introduction.

In quartz [39] it has been shown that protons cause an absorption (determined by the OH stretch) at  $3\text{ }\mu\text{m}$  and careful analysis of crystalline quartz has proven correlations of  $\text{H}^+$  concentration with acoustic Q. Production of high acoustic Q quartz was brought about to a considerable degree because of this characterization discovery.

Trace analysis in inorganic electronic materials has not progressed anywhere as far as that for semiconductors. Perhaps in part because in general properties are not so trace element sensitive, but also due in large degree because matrix interference is often more severe. It is often easier to get consistent relative results with a single method than any sort of reasonable agreement with various methods so as to convince one that absolute accuracy has been obtained. Table 2 showing the results of careful crystalline quartz analysis, brings this point home. An important problem in trace analysis which is often overlooked is that when methods which sample very small volumes of a sample give poor agreement in replicate analysis, the problem may often be unrecognized short distance sample inhomogeneities. An additional requirement for improving most methods is the availability of better standards.

An especially important technological problem where progress is probably at least partly trace analysis limited, is the production of low loss glasses for laser communications. Requirements for losses at optical frequencies of less than 20 db/km will necessitate extremely pure (2 ppb for some transition metals) glasses.

## V. Determination of Physical Structure — Perfection and Defects

The determination of physical structure and perfection should be one of the foundation stones of characterization and begins with the accurate



Table 2. Comparison of analytical methods for crystalline quartz analysis.<sup>a</sup>

Run No.	Doping	Q <sup>b</sup>	Na spectrochem	Na spectrochem. <sup>c</sup> Mellon	Na activ.	Li atomic absorb.	Li spectrochem.	Li spectrochem. Mellon
R27 <sup>d</sup>	0.14M LiF, 0.34M GeO <sub>2</sub> ,							
	0.03M AlPO <sub>4</sub> , 0.5M NaOH	$\sim 1 \times 10^6$	—	—	—	—	665	860
X-0	Natural	$\sim 3 \times 10^6$	—	9	—	—	26	11
X-1	Normal growth	$\sim 1.5 \times 10^5$	94	21	94	23	26	22
X-32	0.14M LiBO <sub>2</sub> +1.0M NaOH	$\sim 3 \times 10^5$	127	42	43-112	60	69	48
X-34	0.14M LiF+1.0M NaOH	$\sim 3 \times 10^5$	44	40	72-127	39	34	60
X-23	0.14M B <sub>2</sub> O <sub>3</sub> +1.0M NaOH	$\sim 9 \times 10^4$	312	238	—	—	—	9
X-16	0.14M LiBO <sub>2</sub> +1.0M NaOH	$\sim 3 \times 10^5$	130	78	—	—	60	129
X-36	0.14M LiOH+1.0M NaOH	$\sim 3 \times 10^5$	<26	18	—	18	—	26
X-26	0.28M LiF+1.0M NaOH	$\sim 4.5 \times 10^5$	<26	18	—	—	26	30
X-35	0.28M LiBO <sub>2</sub> +1.0M NaOH	$\sim 9 \times 10^4$	57	389	—	—	34	29

Table 2. Comparison of analytical methods for crystalline quartz analysis.<sup>a</sup> (continued)

Run No.	Doping	Q <sup>b</sup>	Al spectro-chem.	Al spec-trochem. Mellon <sup>c</sup>	Fe spectro-chem.	Fe spec-trochem. Mellon	Fe activ.	B spectro-chem.	B spec-trochem. Mellon
R27 <sup>d</sup>	0.14M LiF, 0.34M GeO <sub>2</sub> , 0.03M AlPO <sub>4</sub> , 0.5M NaOH	$\sim 1 \times 10^6$	488	666	87	--	--	60	--
X-0	Natural	$\sim 3 \times 10^6$	44	60	78	50	--	--	--
X-1	Normal growth 1.0M NaOH	$\sim 1.5 \times 10^5$	49	35	97	50	870	--	--
X-32	0.14M LiBO <sub>2</sub> +1.0M NaOH	$\sim 3 \times 10^5$	20	8	97	--	87-145	<60	43
X-34	0.14M LiF+1.0M NaOH	$\sim 3 \times 10^5$	16	10	68	--	194	--	--
X-23	0.14M B <sub>2</sub> O <sub>3</sub> +1.0M NaOH	$\sim 9 \times 10^4$	29	27	145	--	--	120	105
X-16	0.14M LiBO <sub>2</sub> +1.0M NaOH	$\sim 3 \times 10^5$	20	8-32	97	--	--	<60	33-47
X-36	0.14M LiOH+1.0M NaOH	$\sim 3 \times 10^5$	11	10	116	few	--	--	--
X-26	0.28M LiF+1.0M NaOH	$\sim 4.5 \times 10^5$	9	7-9-29	97	few	--	--	--
X-35	0.28M LiBO <sub>2</sub> +1.0M NaOH	$\sim 9 \times 10^4$	40	40	135	few	--	180	145

<sup>a</sup> Laudise, R. A., Ballman, A. A. and King, J. C., *J. Phys. Chem. Solids*, 26, 1305 (1965).<sup>b</sup> Q measured at room temperature using precision 5 mc AT-cut resonators.<sup>c</sup> Independent analysis by E. S. Hodge, Mellon Institute, Pittsburgh, Pennsylvania.<sup>d</sup> Prepared at Bell Telephone Laboratory.

determination of structure. The principal techniques used are those of x-ray diffraction. The x-ray techniques applied to perfection are quite different from the more familiar methods used in structure determination. Serious perfection studies almost always involve the use of a wide variety of additional techniques often including etching, decoration, electron microscopy and careful density measurement. Analytical chemists in characterization will find it necessary to be aware of the power and limitations of these techniques. Analytical groups concerned with characterization will often find it worthwhile to include experts with a mastery of one or more of these techniques at their command, and university training programs for analytical chemists may include perfection determination options (especially x-ray techniques). Following some discussions concerning structure, we can do hardly more here than to recite a brief litany of perfection techniques, give some indication of their applicability and provide a few entry references, so as to give the reader a feel for the scope of this often neglected field.

Lattice parameters and symmetry determinations from x-ray powder pictures were discussed above. However, it is important to point out that at the conclusion of a symmetry determination it is only fair to say "Presently, this material has no higher symmetry than that found. Subsequent more subtle tests may reveal lower symmetry." Many x-ray crystallographers fail to realize that for some materials physical tests other than x-ray diffraction are more subtle at revealing symmetry. Optical tests such as birefringence, interference figures, piezoelectric tests with a Geibe-Schiebe circuit and powder second harmonic generation tests [40] ought to be mandatory checks for a lack of a center of symmetry used in conjunction with x-ray investigations. Complete structural determinations are now, of course, greatly accelerated by general availability of automatic diffractometers [41].

In many electronic materials it is important to determine the sense of the electric axis. Two techniques are in general use. The first technique [42] involves the measurement of the integrated intensity of Friedel pairs in transmission through thin plates of the material. A preferable method has been given recently [43] which makes use of the scattering close to an absorption edge of a constituent element where differences in the anomalous scattering are a maximum.

Determination of the sign of an electric axis is an especially ulcer producing occupation. One cannot take refuge in statements or estimates of accuracy of precision. You are either 100 percent right or 100 percent wrong.

Once orientation and structure are established, the next question which often arises is "Is the material a single crystal?", that is, does it contain grain boundaries large enough to limit its usefulness. Grain boundaries and twins may be revealed by optical examination (often between crossed polarizers) by re-entrant angles in single crystals, by etching studies, or by x-ray techniques (commonly the doubling of Laue spots). Grain boundaries are revealed conveniently by Schulz x-ray pictures [44]. The Schulz technique is very much neglected and provides such a straightforward evaluation technique that it should be more widely practiced. Figure 12 shows the geometry and Figure 13 a typical picture. The single crystal is

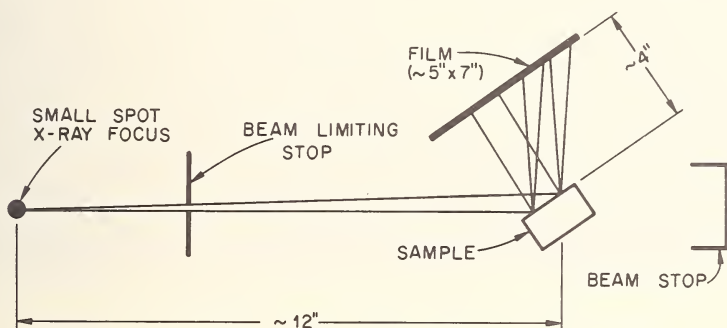


Figure 12. Schulz technique—schematic of apparatus arrangement.

placed at an angle of about  $25^\circ$  to a nearly parallel x-ray beam. White radiation is used. The several spots produced on the film are merely Laue spots, about the same size and shape as the specimen. Each of the spots will be made up of spots of slightly different orientation, each corresponding to a region in the crystal oriented slightly. Resolution is of the order of  $0.1^\circ$ .

In magnetic and ferroelectric materials it is often important to carry out many studies with crystals which contain only one magnetic or electric domain. Magnetic domains are revealed in transparent specimens by microscopic observation in crossed polarizers (Faraday rotation of the polarization depends upon the sense of the magnetic axis). In opaque specimens one is forced to resort to magnetic colloid techniques (Bitter patterns) to observe domains. In these techniques the domains orient small particles of colloidally dispersed magnetic oxides which are smeared on the surface to be examined (or placed close to the surface in



Figure 13. Schulz picture of Verneuil grown sapphire. Spot size and shape correspond to sample size and shape. Approximately rectangular region in spot is misoriented region in sample. Courtesy of R. L. Barns, after Laudise, Reference [4].

a container arranged for easy viewing, *i.e.*, a commercial magnetic tape “reader”). The ultimate method for examining magnetic structure is, of course, neutron diffraction. (See, for instance, Schieber [45] for a review of these techniques.)

The problem of detecting domains in a ferroelectric is analogous. However, birefringence differences brought on by electric dipole reversal are sometimes not observable between crossed polarizers and electric dipoles tend to be easily neutralized by surface charges so that colloid techniques are unreliable. The direct measurement of the pyroelectric constant,  $\alpha$ , is the best way to assure that a given ferroelectric specimen consists of a single electric domain. It will be remembered that  $\alpha = dP_s/dT$  where  $P_s$  is the spontaneous polarization which is related to the magnitude and direction of the electric dipoles. Etching and optical studies can sometimes be used to reveal electrical twinning in piezoelectrics.



At a considerably higher level of perfection, (*i.e.*,  $\leq 10^{14}$  dislocations/cm<sup>2</sup>) dislocations can be studied by:

### *1. X-ray Techniques*

X-ray techniques include the back reflection Berg-Barrett technique; transmission (diffraction) Berg-Barrett technique, Bormann anomalous transmission technique, Lang topographs, double crystal rocking curve photographs, and others. All of these techniques are quite specialized and have been described in the literature so that no detailed remarks are required here. (See Newkirk and Mallett [46] for a review of x-ray techniques used in perfection studies.)

For illustrative purposes we will consider the Lang topograph technique. In the Lang technique a highly collimated narrow beam of x-rays is diffracted through the crystal and the Bragg reflection from the parallel planes approximately normal to the face of the specimen is recorded on the film. The film and specimen are moved simultaneously and a complete "map" of the dislocation network can be obtained. The dislocations are observed as regions of intensified reflecting power through their action in altering the distribution of energy between multiple reflected primary and diffracted beams. The result is that dislocations produce dark regions on the film.

Figure 14 shows a typical Lang topograph. The hardest thing for a neophyte to glean from the literature is what can be expected of each x-ray technique. In Table 3 we have tried to assemble this information. References [4] and [46] should be consulted for "entry" references to each technique.

### *2. Etching Studies*

Etch pits delineate regions of strain and often there is a one-to-one correspondence between etch pits and dislocations. The correspondence between etch pits and dislocations can be shown by a combination of x-ray studies and etch studies by correlation with plastic deformation [47] or a count of etch pits along a low angle grain boundary and experimental correlation of the relationship between pits in the various regions [47]. Etching studies also reveal damage due to surface preparation. Since etching is so easy to perform, it should be considered a standard tool in

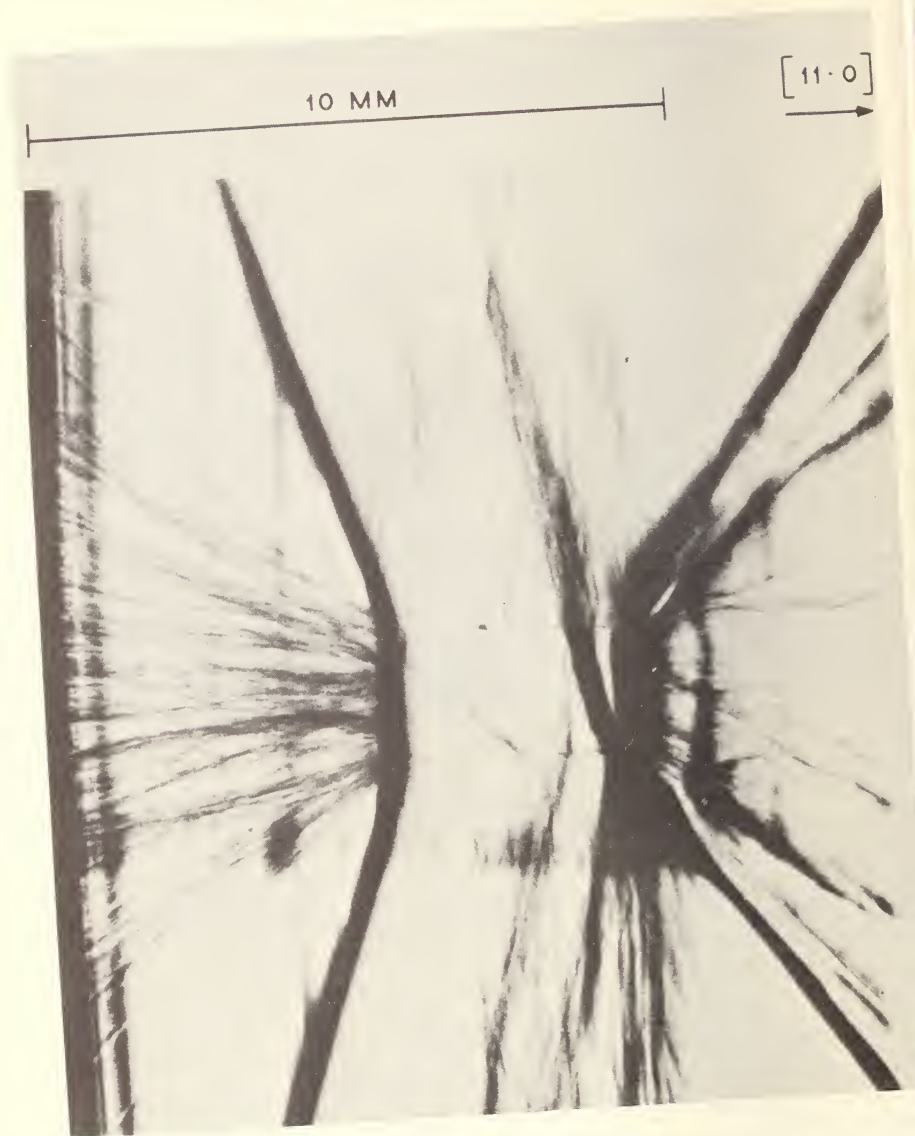


Figure 14. Lang picture of hydrothermally grown quartz. Courtesy of W. J. Spencer, after Laudise, Reference [4].

Table 3. X-ray techniques for studying perfection.

1. Double crystal spectrometer <sup>a, b</sup>	— measures misorientations as small as 0.01 sec. of arc; maps dislocation networks; investigates individual dislocations. Measures lattice spacing variations as small as 0.01 Å.
2. Schulz technique	— measures misorientations of a few minutes to several degrees.
3. Back reflection, Berg-Barrett technique <sup>a</sup>	— maps dislocation networks, investigates individual dislocations.
4. Transmission diffraction, Berg-Barrett technique <sup>a</sup>	— maps dislocation networks, investigates individual dislocations.
5. Bormann Anomalous-transmission technique <sup>a, b</sup>	— maps dislocation networks, investigates individual dislocations.
6. Lang technique <sup>a</sup>	— maps dislocation networks, investigates individual dislocations.
7. Lattice parameter measurements	— measures absolute and changes in lattice parameter and expansion coefficient to 1 ppm. Critical tests of symmetry.
8. X-ray interferometer <sup>a, b</sup>	— measures misorientations of 0.01 sec. of arc; maps dislocation networks, investigates individual dislocations; effective only with rather perfect crystals.

---

<sup>a</sup> Best spatial resolution

<sup>b</sup> Best sensitivity to small strains

the materials characterizer's repertoire (see Laudise [4] for a review of etching and a listing of etches).

### 3. Characterizing Vacancies

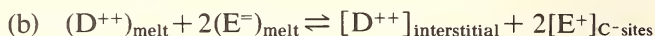
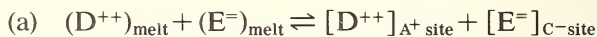
Characterizing vacancies involves the most careful technique and most successful studies have involved correlation of x-ray and pycnometric density. If the vacancy serves as a trap, for instance, the resulting center may be susceptible to optical or electron paramagnetic resonance (EPR) studies.

### 4. Decoration

Decoration, the selective precipitation of a diffusant along low angle grain boundaries and at dislocations is especially useful because it can provide three dimensional maps of perfection (see Lark-Horovitz and Johnson [48] for detailed discussion).

Ion probes, especially when used in channeling studies, show great promise in allowing the direct determination of whether ions are substitutional or interstitial.

Determining whether an impurity is substitutional or interstitial is also sometimes possible by careful density comparisons.<sup>9</sup> Its electrical NMR, EPR or NQR behavior will often be revealing. In many cases the deliberate doping with a particular impurity combined with a careful study of the effect of co-doping with other impurities on its distribution constant may be a convenient means of establishing whether the impurity is substitutional or interstitial. For instance, in the lattice  $A^+C^-$ , if we dope with  $D^{++}$ , which can go either (a) substitutionally at the  $A^+$  site or (b) interstitially, the number of  $C^-$  vacancies will be one for case (a) and two for case (b). If we co-dope with  $E^-$  which is known to go to the  $C^-$  site by the law of mass action for the two cases, we have (for melt growth):



<sup>9</sup> Bearden and Smakula have both considered this method. A more accurate recent value of Avogadro's number, together with Bowman's careful work, on density measurements, have considerably increased recent accuracy. In addition, one must be sure that tabulated isotopic abundances actually apply to the sample at hand [49,50].

For the two cases

$$(a) \quad C_{SD} = C_{\text{melt } D} (E^=)_{\text{melt}}$$

$$(b) \quad C_{SD} = C_{\text{melt } D} (E^=)_{\text{melt}}^2$$

where  $C_{SD}$  is the concentration of D in the solid and  $C_{\text{melt } D}$  its concentration in the melt. This relationship can be easily cast into conventional distribution constant terms.

## VI. Conclusion

Another way of surveying characterization, particularly those aspects which are conventionally within the domain of analytical chemistry, is to list the techniques and state what they can do. Table 4 taken from Reference [3] is a convenient summary arranged in this fashion. Reference [3] also gives a consensus as to limitations of existing techniques. Many strengths and weaknesses of present techniques become apparent from a survey of Table 4.

It is clear that solid state research and electronics has great needs for more and better characterization activity and that analytical chemistry could serve as the core discipline for this activity. At the risk of being at best parochial and at worst outright wrong we will identify those areas which we think are crucially in need of additional support together with some predictions of future directions in characterization:

**1. The development of mapping techniques for sample homogeneity determination should be fostered.** Perhaps it would be more realistic to admit that many crucial problems could be dealt with by present mapping techniques if only researchers and analysts would be sensitized to the need for mapping and willing to apply known techniques. The importance of mapping is readily apparent when we realize that the vitally important economic matter of yield (*i.e.*, percentage of usable devices emerging for a multi-step processing line) in integrated circuits can be related to homogeneity and defect mapping. We can expect here that subtle x-ray techniques will more and more be used as process controls.

**2. The more widespread use of the optical microscope in analytical work is appropriate.** No new techniques are required here. An increased emphasis on the microscope in instrumental



Table 4. Precision and sensitivity of analytical techniques.

Technique	Applications	Sensitivity	Precision (%)
Wet chemistry – titrimetry	Major and minor phase – concentration; also impurities	$10^{-2}$ M in solution $10^{-5}$ M in solution $10^{-6}$ – $10^{-7}$ M in solution	0.01 0.1 0.2–1.0
Wet chemistry – gravimetry	Major and minor phase – concentration	1000 ppm/1 g sample	0.01
Wet chemistry	Major phase – valence		0.01
Coulometry	Major phase – concentration		0.001–0.005
Mössbauer spectroscopy	Major phase – valence also impurities – valence	Down to 0.1% – 0.0001% depending upon density of matrix	Semiquanti- tative now
Electron probe microanalysis	Homogeneity of major phase minor phases	Down to 0.1% over a 1–5 $\mu$ m scan diameter	0.5
Emission spectroscopy	Impurities (survey)	0.1 – 100 ppm	5 – 10
Spark source mass spectrometry	Impurities (survey)	0.01 – 0.1 ppm	Semiquanti- tative now
Atomic absorption	Impurities	0.005 – 0.1 ppm in solution 0.1 – 10 ppm in solution	5 – 10 1 – 5
Flame emission	Impurities	0.002 – 0.1 ppm in solution 0.1 – 10 ppm in solution	5 – 10 1 – 5
Spectrophotometry	Impurities	0.0005 – 0.1 ppm in solution	5 – 10
Polarography	Impurities	All in solution: 0.1 – 1 ppm 10 – 100 ppm .005 ppm 0.001 ppm (with anodic stripping preconcentration)	2 – 10 0.1 – 0.2 20 5 – 10
Neutron activation	Impurities	0.001 – 0.01 ppm	2 – 10
Vacuum fusion – mass spectrometry	Impurities – $O_2$ , $N_2$ , $H_2$	0.7 ppm 100 ppm	20 5
X-ray fluorescence spectrometry	Major and minor constituents	20 – 200 ppm gen- erally; 0.1 at best	0.1

analysis courses would do much to make microscope techniques more widely used. Their use would not solve any unique solid state problems; it would, however, greatly expedite many routine identifications.

**3. Development of new techniques and the more widespread application of known techniques to stoichiometry determination are required.** In solid state technology the 50's and 60's were to a large degree the era of the elemental semiconductors. Binary compound semiconductors are now well established and ternary materials are of increasing interest. The 1970's will see increasing emphasis on binary and ternary compounds, not only for semiconductor research and development but also in nonlinear optical and magnetic applications. As the interest in and commercial importance of compounds grows, so will the need for the accurate assessment of compound stoichiometry. X-ray and hydrostatic density studies, specialized resonance determinations and coulometric analysis should all become of increasing importance.

**4. Reliable methods for trace analysis at the ppb level and below should be developed.** In the 1970's we may expect that electroluminescent lamps will begin to find wider practical application. Electroluminescent efficiency is often dependent upon trap concentration well below the detectability limits of mass spectroscopy. Low loss laser transmission glasses require impurity levels to be controlled in the ppb level. The techniques showing most promise at these levels are activation analysis, coprex and isotope dilution. Increasing attention should and will be paid to these techniques. We may even have to grow accustomed to thinking of mass spectroscopy as a survey technique for relatively dirty materials.

**5. The determination of perfection and defects should become an orderly sub-discipline with its own practitioners.** Presently there are isolated individuals with great expertise in, for instance, the use of particular x-ray perfection techniques. It would be most salutary if some researchers developed a broad interest in perfection assessment. Steps to encourage this development might be taken by means of emphasis on x-ray perfection studies in characterization training, emphasis on perfection studies amongst professional x-ray researchers, and increasing movement of analytical chemists into the area. This field is every bit as big in scope as the chemical analysis part of

characterization and just as essential. It is critically undermanned and should receive every encouragement.

To conclude, what materials characterization truly needs is the active support and encouragement of a cadre of trained professionals. It is my belief that this group of research and applied scientists should nucleate around the analytical chemists. These are the scientists who take pride in sample statistics, to whom ingenuity in deriving new methods is coupled with pride in the painstaking application of tried and true procedures. Couple these attitudes with:

1. An appreciation and knowledge of x-ray techniques which I believe are extremely powerful in elucidating the physical perfection of materials.

2. A familiarity with and willingness to make use of solid state physical measurements such as conductivity and resonance, and

3. A willingness to push reliable trace analysis techniques, especially radiochemistry well into the ppb range.

With this mix of attitudes and techniques I have little doubt that many of the next important developments in solid state-materials science will be catalyzed by the analytical chemist-materials characterizer.

## VII. Acknowledgments

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## PANEL DISCUSSION

### Analytical Problems in Solid State Research and Electronics



#### CHAIRMAN

W. Wayne Meinke, Chief, Analytical Chemistry Division, National Bureau of Standards



#### PANEL

James C. White, Associate Director, Analytical Chemistry Division, ORNL, Oak Ridge, Tennessee

George H. Morrison, Professor of Chemistry, Cornell University, Ithaca, New York

J. Paul Cali, Chief, Office of Standard Reference Materials, National Bureau of Standards, Washington, D.C.

Henry Frankel, Project Engineer, Environmental Technology, International Business Machines, Essex Junction, Vermont

Robert A. Laudise, Head, Crystal Chemistry Research Laboratory, Bell Telephone Laboratories (Speaker)

**Cali** — A large portion of Dr. Laudise's paper was directed toward areas of research in the solid-state that are properly classified as "state-of-the-art." I would like to direct my comments toward the problems faced within the industry itself—the "life on the production line" problems. Over the past several years two major trends in semi-conductor materials analysis have developed.

The first is a marked trend toward topographical analysis. With the advent of large scale integrated devices having device densities approaching  $10^6$  devices per slice, resolution of structure at the micrometer level has become an increasingly more important problem requiring an analytical solution. Three techniques having large potential, especially if used in combination, are in use or in various stages of development. The first is electron microprobe analysis which is, in fact, now widely used, having as it does the ability to determine semi-quantitatively a wide range of elements and structure at the micrometer level of resolution. Its greatest success to the present lies in the determination of defects produced during various stages of the metalization process.

The second technique involves the use of radioactive isotopes to show uniformity of doping. This well-established procedure involves doping the bulk material with the radioisotope of interest, or producing the radioisotope *in situ* by activation, lapping and polishing various subsections, and finally, through radioautography, making visible the spatial distribution of the various dopants in the bulk material. The greatest potential for the determination of topographical features lies in the recently developed ion-probe scanning analyzer. Although the capital investment is very large, the benefits arising from its use warrant its serious consideration for controlling the quality of semi-conductor materials and devices.

The second marked trend in industry is less emphasis on the analysis of the bulk materials than was the case a few years ago. In the early 1960's, major efforts were expended to develop fast, accurate methods adaptable to the quality control of impurities and dopants in the bulk. These efforts, although reasonably successful, have been eased because of the expense involved, and because the reaction time is much too slow for production line quality control. However, the most cogent reason for by-passing these procedures is simply that the "pragmatic approach" works. By this is meant the statistical sampling of representative slices of material measured electrically to provide very fast information as to whether the material is within specifications and should therefore be further processed.

However, this trend may have to be reversed and highly sophisticated

instrumental techniques brought again to the fore. Many of the multi-sandwich layer devices are now produced with yields approaching a few percent or less. Obviously, better quality control of the starting materials, the bulk base materials, and topographical control is required. Three techniques are now widely used. Optical emission spectroscopy using the split-burn technique, developed in part by Professor George Morrison and his co-workers, allows sensitivities approaching the part per billion range. Spark source mass spectrography is a very sensitive method, but one where accuracy is lacking. Accuracy will come about as a wider range of standard reference materials are made available. Radio-activation for many elements is still the most powerful technique, but problems still remain, especially the development and introduction of fast, quantitative procedures for the determination of important light elements, such as H, C, O, and N at the ppb level.

In many of the above mentioned areas, the lack of a Standard Reference Material (SRM) to provide accurate calibration is a serious deficiency. NBS has not been unaware of this need. Unfortunately, SRM's for these purposes are extremely difficult to produce in quantity; the cost of measurement and certification is very high, typically running to \$100,000 or more for one material—say for a completely characterized doped silicon; the market potential is relatively small; and NBS resources for work in this area are limited.

There are, however, several SRM's which fulfill in part some of the needs in solid-state research and technology. There is now available a series of steel SRM's (SRM 661-668) that is extremely homogeneous and can be helpful in the calibration of the spark-source mass spectrograph. Also available is a series of glasses containing 61 added elements in the range from 0.02 to 500 ppm (SRM 610-619). The high purity metals—gold (SRM 685), platinum (SRM 680 and 681), and zinc (SRM 682 and 683) have many elements certified in the ppm to ppb levels and find applicability in the calibration of isotope dilution techniques. Of use for the calibration of the electron microprobe are the Au-Ag wires (SRM 481), the Ag-Cu wires (SRM 482) and the W-Mo alloy (SRM 480).

In preparation is a high purity palladium metal SRM for magnetic susceptibility measurements, and GaAs of known and certified stoichiometry.

**Frankel** — Dr. Laudise cites as an area requiring additional support the development of mapping techniques for sample homogeneity determination which he believes should be fostered. He makes the point that the importance of mapping is readily apparent when we realize that the vitally

important economic matter of yield, that is, the percentage of usable devices emerging from a multi-step processing line and integrated circuits can be related to homogeneity in defect mapping.

Although I agree with the potential benefit which can be obtained from mapping techniques, I believe that there are two formidable obstacles to be overcome. Both obstacles arise because of the complexity of electronic processing steps and manufacture.

To illustrate the problem I would like to go back to the statement that "yield . . . can be related to homogeneity mapping." Device production in general is not based upon perfect crystals. This stems from empirical tests conducted some time ago which indicated that dislocation-free material does not yield more transistors or diodes than silicon with a moderate dislocation density. The reason why dislocation-free material did not yield more good devices than material of moderate dislocation density is process induced defects. So that mapping is but a starting point in a long complex search.

So now we come to our first obstacle—the difference in philosophy and approach of characterization in electronic manufacturing process control as contrasted to the solid state research approach. Research desires to study the relationship of structure and composition with properties. Whereas, in manufacture, the desired properties are known—predominately, if you will, by measuring electrical properties which incidentally also provide a handy comparison scale. Characterization in manufacture is minimal and concerned almost fully with line processing problems. Characterization is thus a court of last resort—when all else fails—try analysis and characterization.

The second obstacle to the characterization analyst arises from the parochial interests of his analytical chemistry past. The range and scope of his work require him to steer a course between the Scylla of studying and characterizing defect for defects sake and the Charybdis of perfecting instrumentation for instrumentation's sake. He can no longer indulge—for long—a pursuit in either direction without suffering in effectiveness to those dependent on his skill. The instrumentation is simply too numerous, the process and physics too complex to permit such unrestrained indulgence.

If we want to reap the benefit of higher yield through characterization, we must intimately incorporate characterization into the process itself. This requires involvement of characterization chemists with the process itself since he must concentrate on line processing steps not only processing problems. He must plan so that he gets most effective information from a sample since most samples are often in no condition to be



tested after electrical test. Incidentally, the "non-destructive" high energy electrons as well as emitted x-rays can and do affect the electrical characteristics of certain critical areas of a device. Planning is also required to select the most relevant test techniques and insure the best use of characterization specialists' time. Lastly, he must foster communication between processing people and characterization specialists in terms of using characterization as a stethoscope to monitor the health of the product.

While I agree with Dr. Laudise that a tremendous amount of information can be achieved through proper use of the optical microscope, I cannot agree that no new techniques are necessary.

As we achieve increasing integration of electronic devices, the working areas available for characterization—now in the micrometer range—will become even smaller.

The tools which can yield analysis of these areas—notably Transmission Electron Microscopy, Scanning Electron Microscopy (SEM) (with x-ray analysis attachment), and Electron Microprobe—will become the most important tools of any device characterization laboratory.

Additionally, surface contamination also becomes of very critical concern. Surface techniques such as Auger Spectroscopy and the Ion Microprobe will become equally important within the next few years.

Now combine all of these "mapping" techniques—spectroscopy, x-ray and microscopy—and we have a formidable armory. In fact, too formidable, since they can and do inundate us with reams of data and information.

I would point out that a single scan at one wave length for a wafer may provide as much as  $2^{19}$  bits of information and a single scan in this case resulting at or near the limits of the optical microscopy in covering only a fifty by fifty micrometer square on the specimen. A ten by ten specimen would require 40,000 such scans at each wave length of interest to acquire all of the data.

It is clearly evident that the computer in the form of image analyzing systems must be included as an analytical tool to decipher, compare, categorize, correlate and recognize defects and patterns. Image analyzing systems with automatic traversals, now primarily encompassing microscopy, should soon be extended to process any raster input including SEM and microprobe.

Lastly, permit me to indulge in what may seem to be a commercial. It seems to me that we analytical chemists while fascinated with the data processing that can be done by computers tied into our GC and x-ray diffraction are but on the first steps of the full use of computers. Consider the



potential of pattern recognition and the use of learning machine concepts to determine relevance of each part of the characterization map. If analytical science is to become the core discipline of materials characterization, we must master these techniques to permit the analytical use of analytical chemists.

**Morrison** — The importance of analytical chemistry to the advancement of materials science over the past two decades is well documented. And conversely analytical chemistry has profited tremendously from its association with solid-state research as evidenced by the dramatic development of new and refined techniques for trace determinations to fulfill the demanding requirements of the study of ultra-pure materials. For instance, spark-source mass spectroscopy was born because of the need for determining trace impurities in silicon and germanium. Activation analysis really got its push because of the need for determining trace impurities at the part per billion level.

Certainly, then the challenges in the past of solid-state research have done well for analytical chemistry. And as Dr. Laudise has pointed out so very clearly, this is only the beginning. There are many more challenges for the analytical chemist in the solving of more sophisticated and complicated solid-state problems.

As has been mentioned, materials science is an interdisciplinary field which deals with the preparation, characterization and relationship of properties to bonding and structure. The three aspects are intimately related. There is one unfortunate thing, however. A problem arises when the respective specialists must interface with each other. This problem exists in all interdisciplinary efforts where the analytical chemist who is technique oriented must interact with colleagues who are problem oriented. And that is what I would like to spend my remaining few minutes on.

Of the various national problem areas being discussed at this symposium, the interfacing on a large scale of analytical chemistry with solid state research has had the benefit of more than twenty years experience. Some of the lessons learned could be of considerable help in developing efficient coupling of analytical chemistry with some of the new national problem areas.

For the past twenty years I have been intimately involved in the analytical aspects of solid state research; ten years in industry and ten years in the university. From my experience the problems of interdisciplinary interaction are the same in both situations.

As Dr. Laudise has pointed out, analytical chemists could serve as the

nucleus for the broader field of materials characterization encompassing identification, determination of major and minor constituents, and the determination of physical structure. The degree of involvement with these many diverse specialties will depend upon the personnel available; however, broadening of the scope should not be done at the expense of diminishing the efforts of the analytical chemist in doing the things he does best, *i.e.* to keep working in the areas of major and minor composition analysis.

In addition to providing service analysis on routine problems, the materials analyst is constantly called upon to develop new quantitative methods for new situations—new matrices, new chemicals, *etc.* Equally important, the analytical chemist must constantly do research on new techniques not directly related to specific materials if he is to continue to be of value in solving new and more complicated materials problems.

Because analysis is a specialty in its own right, requiring considerable expertise, it has been found most expedient in materials research to establish central analytical facilities to handle the many diverse materials prepared by other specialists and whose properties may be studied by still another group of scientists.

At the Cornell Materials Science Center we have a number of central facilities of which analytical chemistry is the largest which provide service to professors, postdoctorals, and graduate students in solid state physics, solid state chemistry, metallurgy, applied physics, electrical engineering and other disciplines engaged in materials research. Different facilities are used to a greater or lesser extent depending upon the materials problem, but analytical chemistry is involved in almost every case.

It has been our experience that the greater the degree of interaction between the various groups, the faster and greater is the chance for success of the programs. We have also found that the analytical chemist, because of his broad scope of assignments, must take the initiative in interacting with the other groups. Thus the analyst must be consulted in the design of a preparation scheme so that sampling for analysis can be optimized thereby leading to more useful data. He must also screen the starting materials. Similarly, close association with those studying the electrical, mechanical, and other properties of the materials is most essential if meaningful correlations with composition are to be achieved.

Although these points may seem obvious to the casual observer, in practice many problems arise because of the lack of communication between the groups. Therefore, those analytical chemists embarking on interdisciplinary programs in the newer national problem areas would do well to heed this lesson.

**White** — I think it is important to look at this problem from the standpoint of the administrator who decides what capabilities existing within his analytical organization can be used to meet the challenges that are presented in solid-state research and electronics. Another point of consideration is which capabilities does he **not** have that can have a considerable impact in this area. In simple language, we're saying — "What do I have available for this problem, and what do I need?" By capabilities I should quickly add that I include manpower as well as instrumentation and equipment. I'd like to pose a few questions that I hope will result in some feedback from you, and offer a few opinions of my own as to what might be a solution to this overall problem of how the analytical chemist can best serve in this area.

What are the characteristics of the analytical chemist who will be of most value to the researcher? What kind of a man are we looking for? He should be a Ph.D. or the equivalent. (Whenever I hear that phrase "or equivalent" I wonder just what is meant by it — other than the fact that this person is probably expected to do everything a Ph.D. does, and know everything a Ph.D. does, but he probably doesn't get paid what a Ph.D. gets paid.) Facetious remarks aside, I do feel that it is highly important that the analytical component of the materials research team be a full-fledged partner with equal status. He must be considered so and of course he has to perform so. Since this field abounds with highly educated, highly trained scientists, he has got to have his credentials. Also he must "speak the language." I think this is vitally important. The successful analytical participant in almost any multi-disciplinary venture always has a good talking understanding of what the overall problem is. In this case he is going to be a materials researcher himself to a certain degree. Fortunately for the analytical chemist, the "language problem" is not as difficult to surmount in this particular area as it is in the biomedical and clinical chemistry area that we will be talking about this afternoon.

Much of the typical training for the typical analytical chemist entails studies that are directly applicable *e.g.* physics, metallurgy, mathematics. So we do know some of the language before we begin. I think it is true to say that we have accepted these disciplines in physics and metallurgy, as proper adjuncts to analytical chemistry. For some unexplained reason, I don't think we feel the same about biomedicine and biology, and I hope we get that changed fast.

To recapitulate then, our analytical input has to be backed by the individual's credentials and an understanding of what the field entails. I think these are qualities that are rather readily come by, but now we face the problem. Dr. Laudise has mentioned the great variety of analytical



techniques, all of which have some useful, and in some cases, even indispensable applications. The analytical man who makes the most significant, visible contribution by necessity will be a specialist—a mass spectroscopist or an activation analyst, or an electron probe microscopist. Unfortunately he can't be a specialist in all of these categories. Thus, we face the likely possibility of overstaffing in a specific category to the detriment of other categories, and create a real imbalance in analytical effort.

The solution obviously is to have several specialists wearing the analytical chemist colors. This is fine if you have these capabilities and can afford to use them, and if your customer can afford to pay you for them. I strongly suspect that all of the required analytical expertise is not going to be available in most laboratories, so the materials research is quite likely to suffer from this imbalance. The mass spectrometer might be used because it's available or because there happens to be a mass spectroscopist "on the team," when activation analysis or polarography might better be employed.

I would venture to say that we as analytical chemists have probably not given the materials field the best possible analytical chemistry available for this particular reason. Nevertheless I think that we will also have to admit that the field has done remarkably well with what has been applied. Much of this credit goes to the materials researchers themselves, who have by necessity become analytical experts in certain specialties. Again, Dr. Laudise has spelled out the opportunity that exists in this field for analytical chemists.

How do we take advantage of this opening? In these times of too many chemists and too few jobs I venture to say that there are not enough analytical chemists who have been trained in performing in the materials science business for the needs of the materials people. I refer to specific type people of course, those people who can assume responsibility for the analytical problems that make a particular input. My proposal of a solution to this problem of specialists centers around what I would term the **analytical broker**.

I think the analytical partner in this partnership should serve the function of advising and probably even more forcefully selecting the particular method to be used for the problem at hand. As was pointed out earlier he cannot be a specialist in all of these techniques. But he is going to have to possess these qualifications I mentioned before and credentials and background about what a technique can do. And he will in turn then communicate the complexity of the analysis to the expert or the specialist who might be employed. I think this man **must** be an analytical chemist.







**George N. Bowers, Jr., M.D.**, is Director of Clinical Chemistry at Hartford Hospital, Hartford, Connecticut. Dr. Bowers majored in chemistry at Colby College in Maine before attending Yale School of Medicine. Internship and medical residency at Hartford Hospital were followed by a fellowship in clinical chemistry with Dr. John Reinhold, Pepper Laboratory, University of Pennsylvania. For the last ten years he has been in hospital service chemistry at the 900-bed Hartford Hospital. Special research areas of interest to Dr. Bowers have included clinical enzymology, ultraviolet and visible spectrophotometry, atomic absorption spectrophotometry, high-purity standards, quality control systems, and high-accuracy referee materials for clinical laboratories. Dr. Bowers is Chairman of the Standards Committee and a member of the Editorial Board of *Clinical Chemistry* of the American Association of Clinical Chemists, and is a member of several groups concerned with the clinical laboratory and medical science fields including the Advisory Panel (NAS-NRC) to the Analytical Chemistry Division of NBS. Dr. Bowers is also actively concerned with environmental issues in Connecticut.



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## CHAPTER 3

# ANALYTICAL PROBLEMS IN BIOMEDICAL RESEARCH AND CLINICAL CHEMISTRY

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The key to progress in national problem areas in health rests over the long term with the success of our country's biomedical research efforts. Analytical chemistry can contribute significantly to these vital efforts by providing the sound base of quantitative chemical measurements required by these health investigations. But health research must not be construed too narrowly. The responsibility for the chemistry related to our national health needs cannot be assigned exclusively to any one scientific group. Indeed, some of the most profound insights may well originate far beyond the usual confines of the medical research community. This will be especially true in the environmental health areas where analytical chemists from many fields will make important contributions.

The elucidation and cure of disease processes are becoming ever more dependent upon chemistry. As a direct result of successful recent past biomedical research, the demand for diagnostic chemistry tests has increased faster than almost any other health service. Service chemistry is thus closely related to medical research and also medical education activities, as will be demonstrated by experience with transaminase assays in heart attacks and calcium determinations in hyperparathyroidism.

To support the rational development and utilization of automated analytical systems in health service and research laboratories, there is a clearly defined need for high purity standard reference materials and certified reagents. Clinical laboratories urgently require the development of high accuracy referee methods to improve the specificity and systematic bias of routine methods.

In short, *life is a chemical system*. Meaningful quantitative chemical measurements are essential to our understanding of its complex internal interactions as well as our profound interdependency upon other life forms in the environment.

Keywords: Biomedical research; clinical chemistry; clinical enzymology; enzyme standardization; health science; hospital laboratory; medical research.

## I. Health, Biomedical Research and Education

“We are determined that the vital link between pure research and practical achievement will never be broken. We are determined that research and discovery yield results which not only increase man’s knowledge but the strength of his body and the length of his life. We do this because we have no choice. And we do it because we believe in Thomas Jefferson’s words, that the care of human life and happiness is the first and only legitimate object of good government.”

Lyndon B. Johnson [1]

In this presentation I will attempt to explore the important relationship which exists between medicine and analytical chemistry. My familiarity with the quantitative chemical needs of medicine is both as a physician trained in internal medicine and as one of three chemists now responsible for chemistry services in a large general hospital. Although these vocational activities tie me closely to the delivery of health care services, I strongly support **biomedical research** as the most logical long-term solution to our nation’s health problems. Thus, in terms of this Symposium’s theme, “**the key to progress in national problem areas**,” the key to this country’s future health rests squarely upon the success of our medical research programs. Analytical chemists can and must contribute substantially to these vital medical research efforts by providing the sound base of knowledge about the complex quantitative chemical measurements which are required in these biological systems.

## A. ANALYTICAL CHEMISTRY AND THE HEALTH FIELD

The health field has many exciting roles for the analytical chemist who enjoys making meaningful measurements. Each day millions of quantitative chemical analyses are required in direct support of individual patient care needs. These service measurements are now one of the mainstays of medicine in the diagnosis and treatment of many human ills. As each year passes, I see more and more chemists finding their way into these vital chemistry service laboratories all across the country. With rare exception, I find these chemists deeply committed individuals who have found great meaning and satisfaction in the chemistry services they provide to others. Table 1 lists the types and numbers of clinical laboratories to show where clinical chemists should be or are now serving in the United States.

Table 1. Clinical laboratories in the United States.<sup>a</sup>

7,000	Hospital laboratories,
4,000	Independent clinical laboratories
615	Federal laboratories
400	State and local laboratories
?	Unknown (nursing homes, unregistered, industrial and insurance)
40,000	Physician office laboratories (each serving less than 3 M.D.'s)

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<sup>a</sup> Estimates by the Center for Disease Control staff in May 1971 during discussions on the need for a National Laboratory Census. Total clinical examinations in 1971  $\cong$  1.4 billion for a cost estimated at \$7 billion.

But health, like chemistry, should not be given too narrow or restrictive a meaning, nor can the responsibility for the chemistry related to our total national health needs be assigned to any one scientific or professional group. Indeed, some of our most significant health related chemical measurements and new medical insights will originate beyond the usual confines of the medical community. As an example, I am reminded that the question of the health effects of environmental contamination by lead was reopened in 1965 by a research geochemist, not by a physician, a toxicologist, a public health scientist, or a biomedical research worker.



In a paper entitled "Contaminated and Natural Lead Environments of Man" [2] Dr. Clair Patterson of the California Institute of Technology presented his reasons for believing that our present-day urban environment increases man's atmospheric exposure to lead by factors of 100 to 1000 times and he speculated about the potential ill effects from this increased exposure on the American people. The point I would make is that this question about health did not arise as the result of the direct study of lead's toxicity to human subjects. Patterson's insight came from a background of years of experience in measuring the relative abundance of nanogram to picogram amounts of stable lead isotopes by mass spectroscopy. His original geochemical research interests established the earth's age as 4.6 billion years; however, later work increasingly centered on the unexpectedly large buildup of lead in ocean surface layers and glacial snows of Greenland [3] as shown in Figure 1.

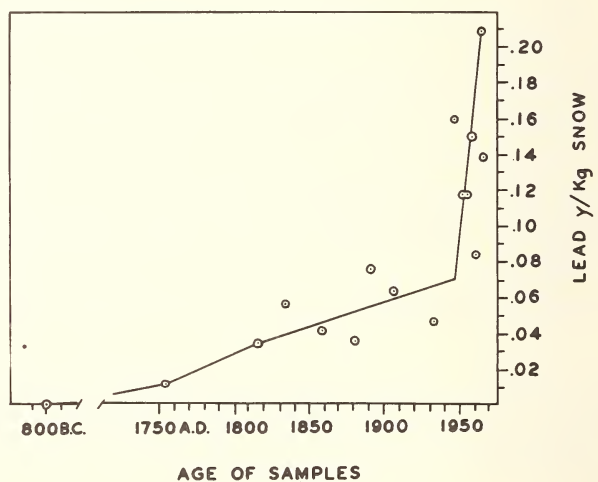


Figure 1. The buildup of lead in Greenland glacier snows correlates well with the industrial use of lead, particularly in recent years with the marked increase in atmospheric lead from gasolines in the United States and Canada.

These studies clearly documented an ever increasing buildup of lead in the northern hemisphere from industrial uses of lead, predominantly from alkyl lead in gasolines. Although many questioned Patterson's arguments about potential health effects, it is interesting to note that Hunt, *et al.* [4] after studying atmospheric cadmium, zinc and lead in 77 mid-western cities in 1968 suggested, "preliminary analysis has shown a positive rela-

tionship between lead dustfall in residential areas of the 77 cities and cardiovascular mortality.”

Thus in numerous ways, observations and meaningful quantitative chemical measurements in each of the major subject areas discussed in this current NBS Symposium will have an important impact upon the health of each of us. Environmental health considerations are coming to the forefront almost daily as we become cognizant that man and his technology are an inseparable part of, neither above nor outside, the natural world. Increasingly we recognize the folly of damaging this complex world on which our existence depends. What is now needed, as Dr. Branscomb, the present NBS Director, has recently pointed out, is that we react with wisdom to these problems created by our technology, using the full power of science to bring about governmental policy “based upon the rational consideration of the scientific facts in their social context” [5].

Can any analytical chemist working in food, water, air, or soil chemistry fail to see the intellectual challenge and opportunities he must address himself to in these complex environmental issues? Certainly in the health field, be it in environmental health, in biomedical research activities or in the medical service areas, our national need for meaningful chemical measurements offers the analytical chemist a remarkable opportunity, in Dr. Meinke's recent words, “to make his contribution and to gain his satisfaction” [6].

## B. BIOMEDICAL RESEARCH

Biomedical research is not a new and novel venture of recent origin. Indeed, from the beginning of recorded history, men have by the rational processes of study and experimentation attempted to control the diseases which crippled and killed them. The pictorial records of ancient Egyptians relieving intercranial pressure by trephining operations or the dietary laws of the ancient Israelites are two examples which come readily to mind.

With the systematic application of scientific knowledge to the study of human ills over the past few centuries, man has progressively freed himself from the ravages of disease. In the last century chemistry has played an important role in our understanding of the fundamental processes of life. For example, while ancient physicians diagnosed diabetes mellitus by the symptom triad of polyuria, polydipsia, and polyphagia and confirmed this diagnosis by tasting urine and/or blood for sweetness, today's physi-



Figure 2. The ancient physician suspected diabetes because of polyuria and confirmed the diagnosis by tasting urine for sweetness. The modern physician makes this diagnosis on evidence from chemical instruments which can detect slight elevations of blood glucose from normal.

cian rarely sees patients so far advanced as to have excessive urination, thirst, or food intake.

Present-day diabetics are often diagnosed on evidence of a slightly elevated level of blood glucose. If there is any doubt, the diagnosis is usually confirmed by obtaining levels of glucose (also insulin in some cases) after carbohydrate loading following careful dietary preparation of the patient for several days. Thus the diagnosis of diabetes mellitus is no longer based on symptoms caused by a disease which is out of control but is now more a differential diagnostic problem to elucidate several chemical aberrations each of which may have altered the normal pathways of energy metabolism. As a result of our increasing knowledge of the abnormal biochemical processes such as the diabetic story just cited, even the layman now recognizes the fundamental chemical nature of life. In short, **life is a chemical process.**

It is not surprising, therefore, to find that each new advance in organic and inorganic chemistry, when applied to the study of man himself, has produced many further insights into the basic chemical nature of health

and disease. By 1900 the introduction of safe systems of public water distribution and the removal of sewerage and refuse coupled with inoculations for smallpox and vaccinations for tetanus had made human existence much less precarious. Also by the turn of the century, the accumulated knowledge of mammalian physiologic chemistry and pathologic biochemistry was already considerable. Steadily over the first several decades of the 1900's biomedical research in chemistry had made important contributions to health with the discovery of cures for or partial control of many nutritional deficiencies and metabolic disturbances, *i.e.*, pellagra, pernicious anemia and diabetes mellitus. However, the chemist and his chemical achievements attracted little public notice since scientists in pathology and bacteriology together with engineers in the public health sciences, were the main contributors to controlling the devastating epidemics of infectious diseases.

By the late 1950's and early 1960's, the record of chemistry related research accomplishments was very impressive. Sulfa drugs had been introduced to control lobar pneumonia in the late thirties, penicillin successfully combated the ravages of syphilis and many other infections in the early forties, powerful anti-inflammatory steroids became available in the late forties, effective drugs against tuberculosis were introduced during the fifties and poliomyelitis vaccines had conquered this dreadful disease by 1960. These accumulated biomedical research and public health efforts had either eradicated or brought under control the impressive number of human ills given in Table 2.

However, in stark contrast with the diseases now successfully managed, the causative factors of those childhood, infectious, degenerative and neoplastic diseases listed in Table 3 are still very poorly understood. Lacking knowledge of their causes they are also not prevented, cured or rationally controlled by human actions. Despite this basic failure, our efforts have not been in vain since the diagnosis and the management of these problem diseases is far more successful today than only a few years ago. These improvements are the direct result of numerous investigations which have increased our comprehension of the specific biochemical functions of each organ and the complex yet measurable chemical interrelationships between the various organs.

Medical research also has been introducing many new anesthetic agents, blood products and immunologic techniques which permit the amazingly intricate new surgical operations of today. Direct surgical attack is now possible for nearly every organ in the human body. Several organs can now be totally transplanted due to the powerful support devices such as heart and lung machines which have been perfected over the past



Table 2. Diseases which biomedical research and public health measures have prevented or controlled by 1970.<sup>a</sup>

## Infectious Diseases

Bacterial Endocarditis	Poliomyelitis	Trachoma
Cholera	Puerperal Sepsis	Tuberculosis
Diphtheria	Rabies	Typhoid Fever
Epidemic Meningitis	Rubella	Typhus
Gonorrhea	Scarlet Fever	Whooping Cough
Infantile Diarrhea	Smallpox	Yellow Fever
Lobar Pneumonia	Syphilis	
Measles	Tetanus	

## Nutritional Diseases

Pellagra	Iron Deficiency Anemia	Scurvy
Rickets	Pernicious Anemia	Dietary Deficiencies

## Metabolic Diseases

Adrenal Malfunctions	Pituitary Problems	Erythroblastosis Fetalis
Thyroid Disturbances	Diabetic Mellitus	Plumbism

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<sup>a</sup> Adopted from an article by P. Handler in *Science* **171**, 144 (1971).

twenty years. In like manner, medical therapy has gained many powerful new chemo-therapeutic substances such as the anti-inflammatory steroids, diuretics, hormones, numerous antibiotics, anti-neoplastic drugs and potent psychopharmacologic agents. Altogether too few of us appreciate how profoundly public health engineering and biomedical research have increased our nation's overall health. There can be no doubt that the quality and longevity of each individual's life in America has improved as a direct result of our past success in medical research.

Today the major efforts of biomedical research in the United States are directed towards developing a comprehensive knowledge of the pathogenesis of these complex genetic, degenerative and neo-plastic processes listed in Table 3. Dr. James Shannon, the former director of the



Table 3. Diseases only partially understood lacking definitive cures and preventative measures in 1970.<sup>a</sup>

## Birth Defects or Genetic Origin Diseases

Birth Injury	Cystic Fibrosis	Muscular Dystrophy
Cerebral Palsy	Hemoglobinopathies	Mental Retardation
Epilepsy	Metabolic Enzyme Deficiencies	

## Degenerative—Chronic Diseases

Arteriosclerosis	Bronchial Asthma	Osteoarthritis
Myocardial Infarction	Multiple Sclerosis	Peptic Ulcer
Rheumatic Arthritis	Senile Psychoses	Regional Enteritis
Systemic Lupus Erythematosus	Emphysema and other COPD	Ulcerative Colitis

## Infectious Diseases

Viral Diseases Without Specific Immunizations	Hepatitis	Mycosis
Acute Rheumatic Fever	Pyelonephritis	

## Neoplastic Diseases

Cancer	Polycythemia	Prostatic Hypertrophy
Leukemia	Dysproteinemia	Uterine Fibromata

## Others

Schizophrenia	Depressions	Alcoholism
Suicide	Mania	Drug Addiction

<sup>a</sup> Adopted from an article by P. Handler in *Science* 171, 144 (1971).

National Institutes of Health, has clearly pointed out that real progress toward prevention, control and amelioration of these chronic diseases rests with the efforts to understand their basic molecular mechanisms [7].

This will require more intensive support for research programs, some of middle range (extending over 5 to 10 years) and others of much longer duration (15 to 25 years in length). These programs must be very fundamental, centering upon the molecular and cellular level of events in normal as well as in pathological processes. If these long-term programs are to be successful, many of the investigators must be young, and dedicated to a long career of inquiry.

It is encouraging to hear our President call for a redoubled effort (\$100,000,000) in cancer research in his 1971 annual fiscal message to Congress. However, the confusion and disruption caused by the drastic cuts in chemistry funding by the National Institutes of General Medical Sciences (NIGMS) as reported in *Science* [8] does not encourage young scientists to race into a life-long commitment to biomedical-chemistry research.

As individuals and collectively as a nation, our future health interests are very closely tied to these long-term research programs. Diverting already limited health research funds into short-term immediate health care services will only delay the time when we obtain the fundamental knowledge needed to solve these chronic disease problems. Rapidly changing priorities in national health goals can only reduce the number of young investigators entering into basic research. Our very real and proper concern for the inadequacies of the present American health care system (or non-system as some are prone to call it) should not rob us of the chance which biomedical research provides of obtaining the knowledge which will ultimately prevent these chronic diseases.

The conclusions of the recent National Academy of Sciences report entitled "Life Sciences" pertaining to the relationship between biomedical research and the development of health care services wisely states ... "We warmly support an enlarged program of research directed at the improvement of our social instruments for the prevention of disease and the delivery of health care. But, equally strongly, we urge that such a program be funded in its own right, without injury to the national biomedical education and research capability" [9].

### C. RESEARCH, EDUCATION AND SERVICE—A MEDICAL TRIAD

To speak of biomedical research as an isolated scientific enterprise divorced from medical education and health care services is unrealistic.

This triad of research, education and service activities constantly interact with one another strengthening the whole body of medical knowledge and practice. Thus, for years it has been recognized that the "best" medicine is practiced when an individual or institution has the benefit of a balanced interaction between all three components as suggested in Figure 3. Medical education together with biomedical research has expanded rapidly over the past twenty-five years. During this time, the National Institutes of Health (NIH) budget which goes predominantly for research and education increased from \$2.5 million in fiscal year 1945 to a recommendation for \$1.9 billion in fiscal year 1972.

While most people associate NIH only with research, the programs to train medical scientists through clinical traineeships and extended fellowships for young medical and doctoral scientists has been a major reason we have had adequate research and education personnel for medical institutions all across this nation. Thus public support of education and research in America has made a long cherished medical ideal of a balanced and dynamic interchange between teaching, research and service, a reality more so today than ever before in history. Despite the recent severe funding cuts in chemistry which have already been men-

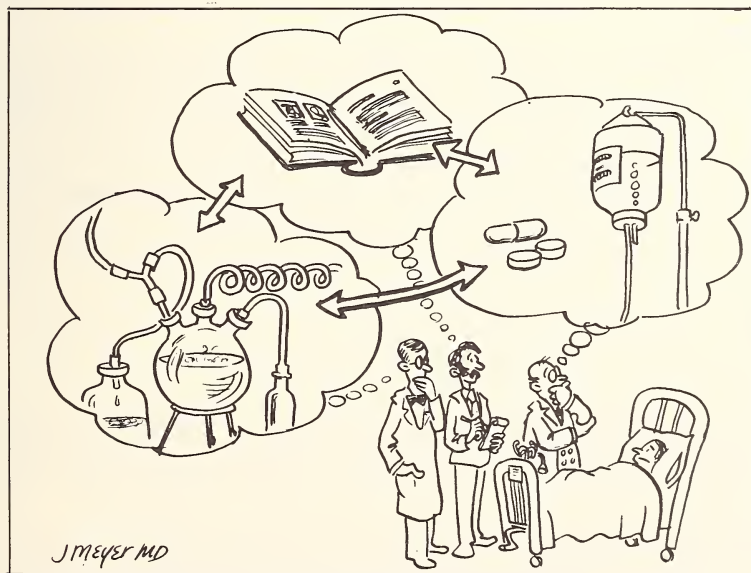


Figure 3. The "best" medicine is practised when there is a balanced interaction between research, teaching and service.

tioned, the nation's present biomedical research and medical education community is the most powerful scientific force dedicated to the advancement of positive human values that mankind has ever known. Or again in Dr. Shannon's words "... the United States now has no peer in the scope and excellence of its medical research activities" [7].

One result of this federal support over the past twenty-five years has been the discovery of literally hundreds of biochemical alterations. A quick review of medical progress reports or special articles from the *New England Journal of Medicine*, or any other broad-coverage medical journal reveals the vast amount of chemical knowledge and its correlation with disease processes which has characterized the past two decades. As a result of the dynamic interchange between research and teaching activities in numerous centers across the country, important biochemical findings rapidly influence medical education. Research-oriented clinicians in medical centers are continuously testing to verify or reject the value of measuring these newly-found biochemical alterations in their patients. Once a measurement has proven to be useful, the information is quickly incorporated into the diagnostic and/or treatment regimen of these clinical investigators. Subsequently through journal publications, lectures and teaching of medical students and house officers, knowledge of the pros and cons of the new measurement diffuses out into the daily practice of medicine.

As a chemist with the responsibility for providing chemistry services for an acute-care one-thousand bed hospital, I can speak with certainty to the pressure for new determinations which medical research and education is constantly creating. Perhaps the success of our past research activities and the effectiveness of medical education which must help translate the new findings into meaningful patient care services can be judged, in part, by the heavy daily usage of these objective scientific measurements.

#### D. QUANTITATIVE CHEMICAL MEASUREMENTS—A RESOURCE ESSENTIAL TO BOTH BIOMEDICAL RESEARCH AND HEALTH CARE SERVICES

Biomedical research and health care activities not only require a tremendous volume of quantitative chemical measurements but also contribute heavily to the development of new methods of analysis. While a few investigators and clinicians may view quantitative chemical measure-



ments merely as "tools," a surprisingly large number recognize how poor measurements limit their work. Many of these research scientists and physicians share with the analytical chemist a deep concern for precision and accuracy.

In parallel with the general trends throughout analytical laboratories today, health scientists utilize instrumental systems of analysis in preference to the traditional "wet" manual methods. Often sophisticated electronic data processing is employed to further increase the speed and reliability of the measurement. As in other areas, limitations of existing analytical methods have repeatedly stimulated the health investigators to improve the accuracy of the measurement and to innovate. Both research and service needs have resulted in unique innovations in measurement systems to solve specific problems. An outstanding example of such a recent innovation is the development at Brookhaven and also simultaneously in England of whole-body neutron activation analysis which permits the *in vivo* study of skeletal calcium metabolism in patients suffering with osteoporosis [10]. Likewise in the clinical service area, the original Auto-Analyzer® developed by Skeggs represented an inventive means to solve the problems of heavy daily workloads in the face of inadequate numbers of skilled personnel [11].

This relationship between instrumental measuring capability and improved biomedical research or health care services is easily seen in a review of equipment found in either investigative or clinical laboratories today in comparison with twenty years ago. In 1950 a few filter colorimeters, a simple pH meter and a spectrophotometer were all that was required to be a reasonably well equipped laboratory. However, in 1971 capital equipment plays a far greater role in the plans of any research or service laboratory. While the instrument needs in research will vary greatly depending upon the project, many of the vital services a clinical chemistry laboratory provides today would be utterly impossible without the significant advances in analytical instruments which have occurred. One might cite the contributions of the flame photometer for rapid and accurate sodium, potassium or lithium measurements. Certainly the advent of atomic absorption spectrophotometers has improved measurements for calcium and magnesium while also introducing the ability of testing for trace metals such as zinc, chromium and copper or the toxic elements—lead, mercury, cadmium and arsenic. Although we could mention several other important instrumental contributions in this section, I would now like to show how the advent of one modern instrument, the pH, pCO<sub>2</sub> and pO<sub>2</sub> meter with its capillary type electrodes and reliable electrometer, has had a profound impact upon improving teaching and patient care.



## E. ACID-BASE AND BLOOD GAS MEASUREMENTS

Until 10 years ago, the only test available to judge the body's acid-base balance, even in the largest medical centers, was the plasma bicarbonate or the slightly more refined but qualitatively similar  $\text{CO}_2$  content. Since it is possible for a high blood  $\text{CO}_2$  to be found associated with either alkalosis or acidosis much confusion was possible as illustrated so vividly by Dr. Meyer in Figure 4. Biomedical researchers such as Henderson [12] and Van Slyke [13] had almost completely described the physiologic relationships and controlling mechanisms involved in acid-base balance as well as alterations due to pathologic processes many years ago during the 1920's and 1930's. However, only a few clinicians were able to obtain pH and  $\text{pCO}_2$  measurements on patients. For example, during my fellowship training in clinical chemistry in 1958 at a major university medical center, these measurements were usually made only by the most senior doctorate chemist. Reliable measurements by technical people were unheard of because the instrumentation was so difficult to operate correctly.

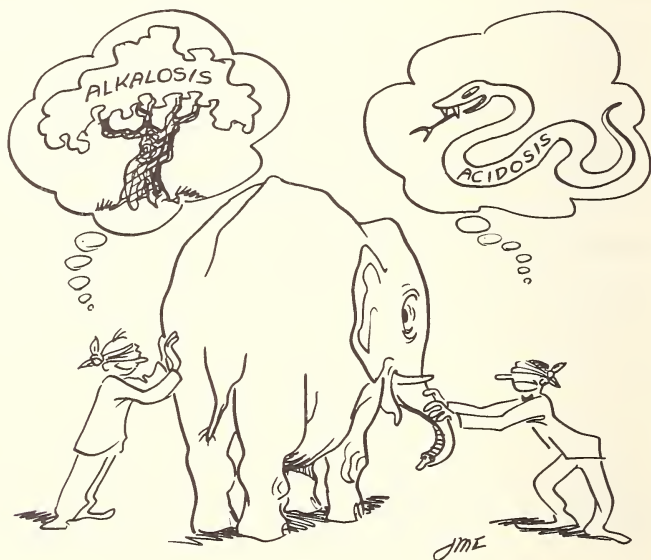
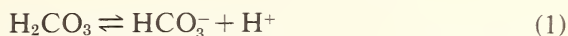


Figure 4. An elevated blood  $\text{CO}_2$  alone without pH and/or  $\text{pCO}_2$  measurement could mean either acidosis or alkalosis. The clinician was forced to use "clinical-judgement."

In medical schools the subject of acid-base balance was usually taught mainly by renal physiologists. The major emphasis was often on the interpretation of bicarbonate measurements using equations which involved assumptions of a fixed respiratory response. The kidneys' regulatory role by virtue of its excretion of hydrogen ion by various exchange mechanisms was stressed (see Equation 1).



Few students or clinicians fully appreciated how rapidly the excretion of  $\text{CO}_2$  by the lungs could affect acid-base balance although this was well known by respiratory physiologists and anesthesiologists.

The ability to measure pH,  $\text{pCO}_2$  and  $\text{pO}_2$  quickly and reliably on patients with respiratory and/or circulatory problems has now shown how rapidly alterations in alveolar gas exchange will alter the body's acid-base homeostasis. The lungs' role in acid-base balance by ventilatory control of  $\text{CO}_2$  is expressed by Equation (2).



Thus the regulation of acid-base balance is the result of two distinct and separate control mechanisms, one slow process of renal hydrogen ion excretion and another rapidly responding process of respiratory  $\text{CO}_2$  excretion. Together these provide hydrogen ion homeostasis by Equation (3).



Today good laboratory support allows even the medical student or young house officer to make more accurate evaluations of a patient's acid-base status than the most senior consultant in metabolism was capable of doing 10 years ago. Despite the inherent complexities of whole blood pH measurements, modern instruments perform remarkably well and the chemist rarely needs to confuse his clinical colleague with highly technical problems as suggested in Figure 5. The following two cases illustrate what a difference pH,  $\text{pCO}_2$  and  $\text{pO}_2$  measurements can make [14].

**Case Number 1** A 63-year-old woman with a long history of pulmonary disease characterized by frequent infections, pneumonia, chronic productive cough and chest cage deformity was admitted to the hospital due to one week of crampy abdominal

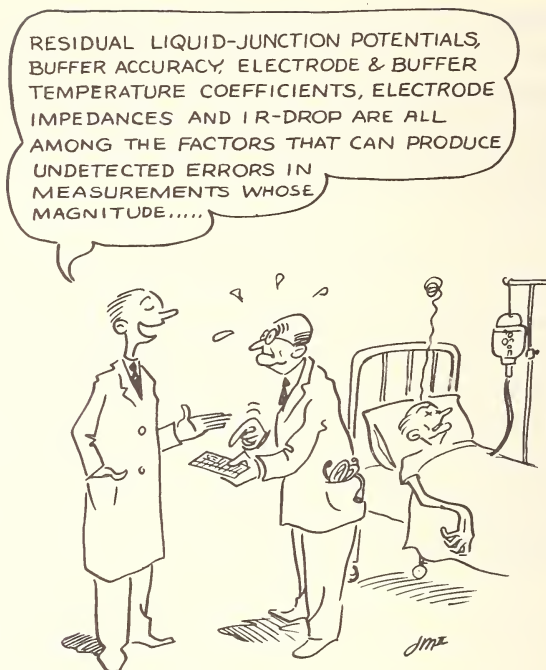


Figure 5. Modern pH instruments perform remarkably well. The clinical chemist rarely needs to confuse his medical colleagues with the inherent complexities of whole blood pH measurements.

pain, nausea, vomiting and mild diarrhea. Physical examination showed only diffuse abdominal tenderness and marked kyphoscoliosis. All laboratory tests were normal except  $\text{CO}_2$  content of 34 mmol/l (normal 18-24 mmol/l). No pH or  $\text{pCO}_2$  measurements were made. **Progress:** The surgeon felt that  $\text{CO}_2$  elevation and history of vomiting suggested metabolic alkalosis (loss of HCl in vomited gastric fluid). The patient was treated with ammonium chloride as preparation for surgery and four hours later was unresponsive, cyanotic, hypotensive and air hungry. At this time pH was 7.1 and  $\text{pCO}_2$  65 mm Hg and  $\text{CO}_2$  content had dropped to 21 mmol/l. The patient died several hours later! **Interpretation:** This patient's initial acid-base problem was respiratory failure with carbon dioxide retention.

The administration of ammonium chloride converted a metabolic compensation into a severe metabolic acidosis on top of an underlying problem of respiratory insufficiency. If either the pH or the  $p\text{CO}_2$  in addition to the  $\text{CO}_2$  had been obtained, this lady's precarious respiratory status would have been recognized and not compounded.

**Case Number 2** A 40-year-old fireman was stabbed in the chest with injury to pulmonary artery, aorta and right ventricle of the heart. In the Emergency Room his heart stopped but responded to external massage. The heart and great vessels were repaired and the patient responded well after blood transfusion. During the ER resuscitative efforts gastric contents was aspirated into the lungs with resulting severe bilateral pneumonitis. For several days the patient's condition was critical; despite oxygen and steroids he remained deeply cyanotic and air hungry. On the fifth day he was much worse. Respiratory acidosis was clinically suspected since the  $\text{CO}_2$  content was 40 mmol/l. However, before alkali was administered a venous pH was obtained which was 7.61 (severe alkalosis) while his  $p\text{CO}_2$  was only 45 mm Hg. In retrospect, this severe metabolic alkalosis was due to the constant removal of HCl from the patient's stomach by a Levin tube. Treatment with intravenous fluids containing potassium, resulted in prompt clinical improvement and return to normal acid-base balance in 48 hours. His subsequent course was one of continued improvement. **Interpretation:** This case might well have had a fatal outcome had the pH and  $p\text{CO}_2$  not been obtained prior to giving treatment.

Both of these patients were seen during the very early days of the introduction of blood gases and pH measurements to our hospital when clinicians did not always recognize the valuable help such tests gave. Today **Case 1** would never be evaluated by  $\text{CO}_2$  alone and the happier outcome depicted in Figure 6 might have occurred. Our present goal is to perform and report back to the clinician all pH,  $p\text{CO}_2$  and  $p\text{O}_2$  measurements within 20 minutes of the time it reaches the laboratory. In the near future this needs to be reduced to 5-10 minutes; however, membrane problems seem to limit the long-term electrode stability and standardization must precede each whole blood measurement at present.

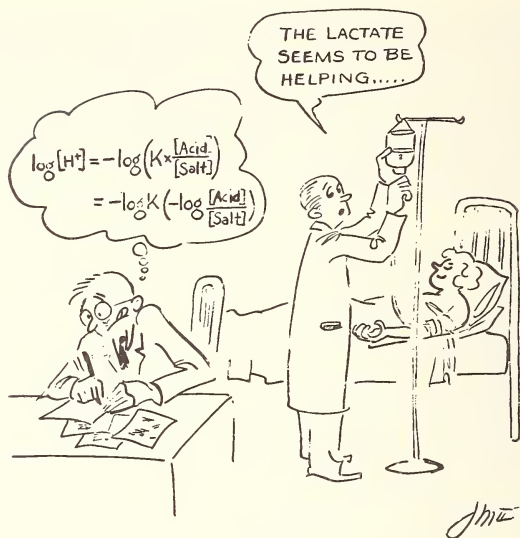


Figure 6. Properly applied pH, pCO<sub>2</sub> and pO<sub>2</sub> measurements can restore the smile-of-health to many a patient.

## II. Clinical Chemistry—Analytical Chemistry in Medicine

“The clinical chemistry laboratory a few decades ago was a modest handmaiden supplying a few chemical tests that appeared serviceable in diagnostic procedures ... A great change was inaugurated when a few progressive hospitals appointed to their laboratories highly trained professional chemists, men who were qualified to carry on investigative work in addition to supplying reliable data for diagnostic use. These chemists faced great challenges in refining existing methods and in the development of a rapidly increasing number of new tests and techniques.”

Somogyi [15]

No part of clinical medicine, even the much publicized hospital emergency room services, has grown and changed more in the last decade than clinical chemistry. This rapidly changing nature of clinical chemistry services in any large institution today can best be understood by contrast with the same laboratory in 1950.



## A. QUANTITATIVE AND QUALITATIVE CONSIDERATIONS

Quantitatively, the total volume and types of tests performed have steadily increased each year requiring more space, instruments and personnel. Qualitatively, to meet articulate clinical demands, the services have had to be more rapid with an ever greater degree of proven reliability. The following description drawn from my past twelve years of experience at Hartford Hospital will attempt to provide the chemist who is unfamiliar with the practice of clinical chemistry (analytical chemistry in a medical environment) some idea of its functions and services. One will readily recognize that manual manipulations of test tubes, Bunsen burners, and visual comparative colorimeters now belong to a past era.

### *1. The Volume and Types of Tests Performed*

The total volume of objective measurements requested of the entire laboratory at Hartford Hospital has risen progressively since 1950 as shown in Figure 7 on the top curve. Chemistry services, as seen by the lower curve of Figure 7 have increased at an even greater rate (going from 100,000 tests in 1959, to 190,000 in 1964, 377,000 in 1969, 430,000 in 1970 and to an estimated 485,000 in 1971). This doubling in volume every five years is typical of the demands made upon clinical chemistry laboratories in nearly all hospitals in North America and Western Europe. Although it is difficult to judge just what the future growth trends will be, there is at present no reason to expect this twenty-year pattern to suddenly reverse itself. Therefore, we currently are making our plans on the basis that this laboratory shortly after 1975 but before 1980 must provide 1,000,000 service chemical determinations yearly.

Only part of the increase in test volumes can be ascribed to greater use of the thirty tests available in 1950 as listed in Table 4. The effect on total volume of introducing fourteen new tests while only dropping four old tests between 1950 and 1960 was especially marked because of the addition of the two transaminase enzymes which will be discussed in greater detail later. Between 1960 and 1970 thirty-five additional tests were offered but in just the last eighteen months approximately thirty new types of tests have been added. Today, some one hundred discrete types of chemical tests are available routinely whenever the physicians wish to initiate an order for them. Appendage A lists a breakdown by laboratory sections of the 1969-70 volume and the observed precision (expressed as

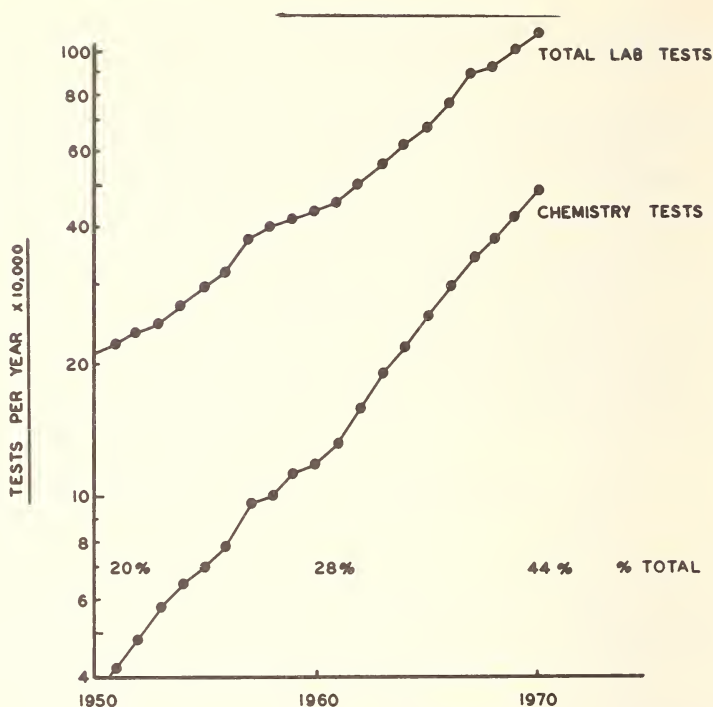


Figure 7. The growth in clinical chemistry (bottom) is contrasted to the total laboratory tests for the period 1950 to 1970. Note that chemistry services have gone from 20 percent of the total tests in 1950 to 44 percent in 1970.

the Percent Relative Standard Deviation (% RSD) for  $n = 80$  to 120 days). Appendage B lists the tests not performed in our laboratory as of June 1971; these are sent out to several outside reference type laboratories.

## 2. Organization and Personnel

Until July 1959, the scientific direction of clinical chemistry at Hartford Hospital was shared by five general pathologists with no single individual specifically assigned to manage the section. A medical technologist-supervisor guided the day-to-day operations of nine medical technologists who easily handled the total yearly workload of approximately 100,000 tests by forty different methods. Emergency tests were limited to blood sugars,

Table 4. Tests added and revised in clinical chemistry  
(Hartford Hospital experience 1950–1971).

OFFERED PRIOR TO 1950  
(30 tests)

Blood sugar***	Chloride**
(Non-protein nitrogen)	Carbon dioxide
Uric acid*	Cephalin flocculation
Creatinine*	Thymol turbidity*
Creatine	BSP retention*
Cholesterol**	PSP excretion*
(Icteric index)	(Sulfa levels)
Bilirubin*	Salicylates*
Total protein*	Bromide
Albumin*	Protein bound iodine**
C.S.F. protein*	Amylase
Acetone	Lipase*
Calcium*	Acid phosphatase*
Phosphorous*	Alkaline phosphatase**
Sodium***	(Sulkowicz)
Potassium***	Renal calculi**

ADDED PRIOR TO 1960  
(14 new – 4 dropped + 30 = 38 tests)

Serum electrophoresis '52**	SGPT Transaminase '58*
Hemoglobin S '52**	Tubular PO <sub>4</sub> Reabsorption '58
Sweat test '53*	Urea '58*
Cholinesterase '56*	Quality control '58*
Atypical cholinesterase '57*	Microbilirubin '59*
17-ketosteroids '57	Methemoglobin '59*
Serum Fe + TIBC '58*	Plasma Hemoglobin '59
SGOT Transaminase '58*	

ADDED PRIOR TO 1970  
(35 new + 38 = 73 tests)

Lactic dehydrogenase '60*	C.S.F. electrophoresis '65
Magnesium '60*	Prostatic acid phosphatase '66
Barbiturates '60*	Macroglobulin '66
Ethanol '60**	Amino acids '66
Blood ammonia '61*	Urine sugar – TLC '66
Ceruloplasmin '61	Plasma cortisol
Carotene '61	Blood pH '67*
17-Hydroxysteroids '61	Blood pCO <sub>2</sub> '67*

Table 4. Tests added and revised in clinical chemistry (continued).

VMA '62**	Blood pO <sub>2</sub> '67*
Hemoglobin electrophoresis '62*	Amniotic fluid '67*
A <sub>2</sub> Hemoglobin '62**	Lithium '67
Bicarbonate '63	Triglyceride '68
5 Hydroxy-indole acetic acid '63*	Galactose '68
Osmolality '63*	Tolbutamide Top. '68
Xylose '64	Urine Lead '69
Doriden '64*	Sperm Fructose '69
Urine electrophoresis '65*	Creatine Kinase '69

## ADDED TO PRESENT (June 1971)

(30<sup>+</sup> new + 73  $\cong$  100<sup>+</sup> tests)

Metanephrine '70	Mercury '71
Blood lead '70*	Blood volatiles '71
Tissue lead '70	Drugs by TLC '71
Copper '70	Dilantin '71
Zinc '70	Ionized calcium '71
Lipoprotein electrophoresis '70	Uroporphyrin '71
Immunoelectrophoresis '70	Coproporphyrin '71
Immunodiffusion for IgA, IgM, IgG and fetoprotein '70	Porphobilinogen '71
10 Microchemistry tests '70	Urobilinogen '71
6 Drugs by GLC '70	Melanin '71
Complement '71	Quant. urine proteins '71
Placental Estriol '71	Pregnanetriol '71
Placental (heat stable)	Lactic dehydrogenase
Alkaline phosphatase '71	Heat stable '71
Cadmium '71	Glucose - enzyme specific '71

NOTE: Asterisks indicate number of revisions.

Parentheses indicate tests which were discontinued.

urea, acetone and salicylates which were performed, when regular personnel were unavailable, by residents and college students who also covered the entire laboratory including the blood bank. The space allocation to chemistry to provide these services in 1959 was 1500 ft<sup>2</sup>.

In contrast to that small, loosely organized but well-functioning laboratory of July 1959 mentioned above, the clinical chemistry laboratory of July 1971 has evolved into a highly-structured grouping of five sections as shown in the organizational chart in Figure 8. The former single produc-

## CLINICAL CHEMISTRY LABORATORY -- ORGANIZATION CHART

(Hartford Hospital, June 1971)

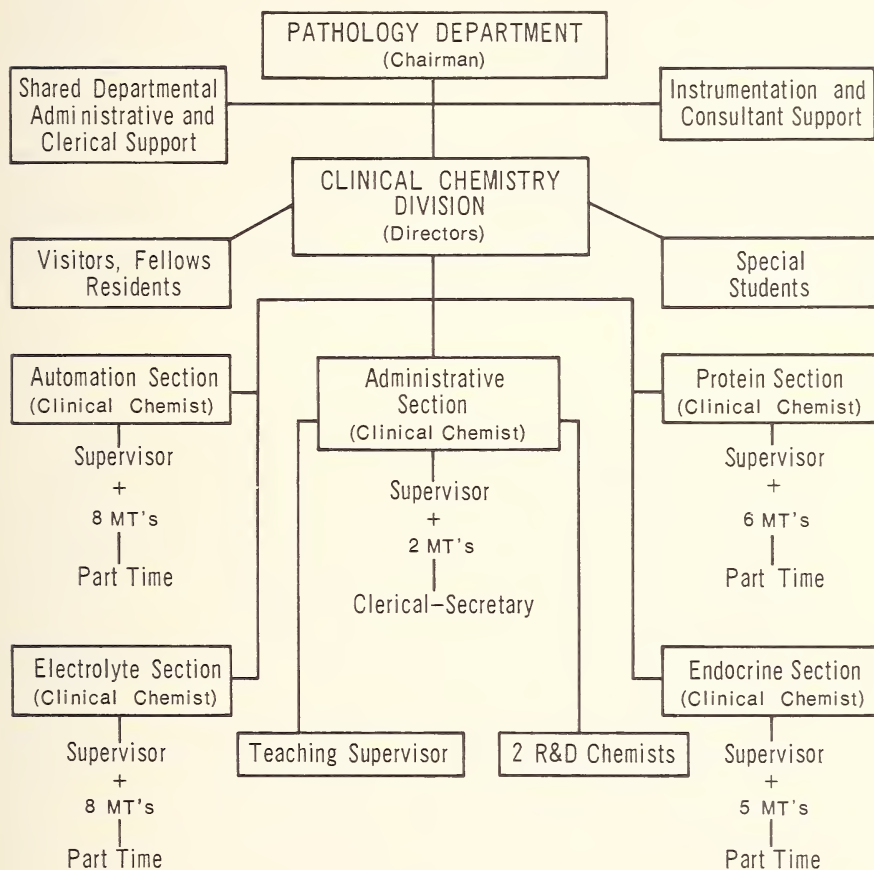


Figure 8. Organizational graph showing the structure of clinical chemistry in a 1000 bed acute care community hospital.

tion section has become four (automation, electrolytes, proteins and endocrines) and a fifth off-line administrative section combines the functions of clerical-secretarial support, standards and controls, research development, education and overall division management. The personnel number 49 individuals as follows: one M.D. chemist, one Ph.D. biochemist, one Ph.D. analytical chemist, one Ph.D. post-doctoral fellow, one instrument specialist, two developmental chemists, five supervising technologists,



one teaching supervisor, 36 medical technologists, two clerk-secretaries and six full-time technologist equivalents (20 part-time persons) covering nights, weekends and holidays. In addition, some 15 clerical and management persons are shared in common with the other clinical pathology divisions and four doctorate persons (two M.D.'s, one biochemist and one physicist-instrument designer) provide various consultative support services.

To give another way of viewing the diverse skills and talents of the organization, the technical, chemical, and medical competences of the present chemistry organization are listed in Table 5. Chemistry space requirements have also changed with approximately 4,000 ft<sup>2</sup> in 1969-70 and within the 1972-73 hospital fiscal year new construction will increase this to approximately 12,000 ft<sup>2</sup>, which should be adequate until 1980.

Table 5. Competencies in Clinical Chemistry (Hartford Hospital Laboratory, June 1971).

I. Optical Methods	IV. Other Instrumental Methods
Spectrophotometry	Refractometry
Spectrofluorimetry	Osmometry
Flame photometry	V. Classical and Other Methods
Atomic absorption	Gravimetry
II. Electroanalytical Methods	Solvent Extractions
pH, acidity and blood gases	Titrimetry
Potentiometry	Ultramicrochemistry
Coulometry	Standards
Conductance	Quality Control Materials
Ion specific electrodes	VI. Skills
III. Chromatographic Methods	Analytical - electrochemistry
Paper chromatography	Analytical - biochemistry
Thin layer chromatography	Enzymology - kinetics
Gas chromatography	Toxicology
Ion exchange	Internal Medicine
Electrophoresis	Environmental Health
	Protein chemistry

Table 5. Competencies in Clinical Chemistry, (Hartford Hospital Laboratory, June 1971) (continued).

#### VI. Skills (continued)

Spectroscopy (AAS)	Teaching skills
Computer programming	Administrative techniques
Statistical – control methods	Medical technology skills
(Pathology—Gross and Micro)	Immunology and genetics
Instrument fabrication	Editorial and Publication
Poisonous mushroom	services
identification	Reagent water purity

### 3. Instrumentation

Without any doubt, the most profound change which has occurred since 1950 in hospital laboratories of this country is the use of instrumental, to replace manual methods of analysis. In 1958 the chemistry laboratory acquired its first single channel semi-automated blood analyzer (Technicon's AutoAnalyzer<sup>®</sup>). At that time the major dependency on instruments rested on either Klett double beam filter colorimeters or the Coleman single beam simple grating spectrophotometers of wide band pass. Although, as shown in Table 6, absorption spectrophotometry (now of significantly better photometric performance) is still the mainstay of the clinical chemistry laboratory, the steady introduction of other types of instruments is striking. The percentage of total tests performed by each class of instrumental methods can be expected to change in future years from those given in Table 6, especially as electroanalytical and gas chromatographic methods become more firmly established in hospital service laboratories. I would also predict that liquid-liquid chromatographic methods of analysis will be utilized more fully to resolve complex mixtures into readily measurable fractions hopefully without extensive preparatory scrub-up procedures [16, 17].

By the criteria of some clinical laboratory scientists, an inspection of Table 6 would suggest that our laboratory at Hartford lags in the use of highly automated instrument systems and "on-line" computer equipment. Both systems have become very common in hospital and private clinical laboratories all across the country and will certainly play an ever increas-

Table 6. Methods and instrumentation (Hartford Hospital, June 1971).

## I. Optical Methods and Instruments – 91% of total service testing

## A. Ultraviolet–visible absorption 68%

1. Colorimetry (interference filter photometers) – 42%
  - a. 9 single channel autoanalyzers (181,000 tests)
2. Simple spectrophotometers (wide band pass) – 8%
  - a. 3 B & L Spec 20's (service)
  - b. 4 Coleman Jrs. (teaching)
  - c. 2 Kletts (teaching)
3. Small spectrophotometers (narrow band pass) – 2%
  - a. 3 Gilford 300 N's
  - b. 1 Beckman B
4. Enzyme – kinetic instruments (narrow band pass) – 16%
  - a. 2 Gilford 2000's with Beckman DU monochromators (71,000 tests)
5. Recording spectrophotometers – 1%
  - a. 2 Perkin–Elmer 202
  - b. 1 Beckman DK-1
6. High performance spectrophotometer – 0.1%
  - a. Cary 16 for standardization work

## B. Flame Photometry (79,000 tests) 18%

1. Types of instruments
  - a. Instruments Laboratories Inc. Model 143 b (service)
  - b. 2 Baird KY-1 (teaching)

## C. Atomic absorption spectrophotometry (14,000) 3%

1. Types of instruments
  - a. 1 Instrumentation Laboratories Inc. Model 153
  - b. OCLI Instrument AAA-3 (loan)

## D. Fluorimetry &lt; 1%

1. Turner Model No. 110
2. Aminco–fluorimicrophotometer

## E. Infrared spectrophotometry 0.1%

1. Perkin–Elmer-700

Table 6. Methods and instrumentation (continued)

- II. Electroanalytical Methods and Instruments – 4%
- A. Blood pH,  $p\text{CO}_2$ ,  $p\text{O}_2$  systems
    - 1. 3 Corning blood gas Model 160
    - 2. 2 Radiometer PMQ 4
  - B. Other pH equipment
    - 1. 2 Orion Model 401
    - 2. Beckman Model G
  - C. Coulometers
    - 1. 2—Cotlove chloride titrators
  - D. Conductivity Meters for Reagent Grade Water Testing
    - 1. Radiometer, Model CDM 2
    - 2. Constant recording unit from Foxboro—Model 9460 AC/PC Dynaloy Recorder C with Beckman conductivity cell (cell constant = 0.001)
  - E. Specific Ion Electrodes
    - 1. Chloride – Orion sweat testing Model #417
    - 2. Calcium – Orion calcium ion-specific electrode Model 99–20 and Model 801 meter
- III. Electrophoresis Chromatography Methods and Instruments – 3%
- A. Protein electrophoresis units and densitometers
    - 1. 2 Beckman Microzone Systems R101 with densitometers
    - 2. Starch gel apparatus – Buckler
    - 3. Immunoelectrophoresis – Shandon
    - 4. Lipoprotein electrophoresis – Shandon
  - B. Thin Layer Chromatography Apparatus – Buckler
  - C. Gas Liquid Chromatograph with flame ionization – Hewlett Packard Co., Model 5750B
- IV. Other Instrumental Methods  $\cong$  1%
- A. Protein by refractometry
    - 1. American Optical Co. TS Meter Model 10400
  - B. Osmometer
    - 1. 2—Advanced Instruments Inc. Systems
      - a. Model #3D
      - b. Model #64–31

Table 6. Methods and instrumentation (continued)

## V. Manual Methods &lt;1%

- A. Flocculations
- B. Immunodiffusion quantitation
- C. Titrimetry

## VI. Ancillary Equipment

- A. Electronic Data Processing Calculators
  - 1. Olivetti Underwood Programma 101
  - 2. Monroe Desk Top Calculator Model #820
- B. Computers – (limited access use)
  - 1. Digital Equipment Companies – PDP 15 (quality control data)
  - 2. IBM 360 (hospital based) – (billing, inventories, budgets)

ing role in providing chemistry services in the future. We have deliberately waited on the use of highly-automated equipment because of reservations about flexibility, standardization and accuracy. Yet at times one must admit to feeling somewhat confused by the swift changes in both the economics of laboratory medicine and the growing technological complexity of the alternate instrumental choices. My pathologist friend Dr. Meyer has again caught something of this in his cartoon in Figure 9 which was used to illustrate an article by Alpert entitled, "Automated Instruments for Clinical Chemistry: Review and Preview" [18].

*4. The Clinical Chemist's Role in the Medical Community*

The central scientific responsibility of a hospital clinical chemist is to bring the **full power** of reliable quantitative measurements to the patient. Although he must always provide his analytical work within the constraints of clinically determined time limits, valid measurements, not rapid answers, must be the goal. Precision and accuracy in clinical chemistry are dependent upon (1) the standards and (2) the method. As in any analytical technique, aqueous standards must be used concurrently and span the entire range of values found in the patient's specimen if error is to be minimized. Just as the former practice of using factory precalibrated





Figure 9. One must admit to feeling perplexed at times by the swift changes and growing technological complexity of analytical instruments for clinical laboratories.

photometers without frequent calibration violated sound analytical practices, so does single point calibration of instruments with normal levels of serum reference materials which bypass the long-proven standardization concepts upon which accurate measurements must rest. As always, acceptable methods, be they manual or instrumental, must be judged in terms of sensitivity, precision, specificity and accuracy.

Once the accuracy of the analytical measurements has been assured by proper use of standards and precise methods of low systematic bias, the clinical chemist must then pay attention to the special "people problems" given below.

**a. Sampling.**—Although the preparation of patients, their diets and the timing of service requests are primarily the physician's responsibility, the chemist must do everything he can to be sure a correct sampling is made under conditions which later permit the physician to interpret the test. Life is a dynamic process with many involuntary changes (respiration, diurnal rhythms, *etc.*) and voluntary changes (*i.e.*, meals and activity). Although completely physiologic, these normal processes can nevertheless invalidate the finest measurement if ignored. Dr. Meyer in Figure 10 has strongly suggested that one must be sure that all laboratory personnel act with intelligence and concern in obtaining specimens. Sampling and handling are as important as any of the subsequent phases of separation, isolation, measurement and calculation. It has been my experience that sampling errors during specimen procurement, labelling or transporting, coupled with mistakes in reporting have been far more frequent than measurement errors since the introduction of quality control programs as suggested by Figure 11.

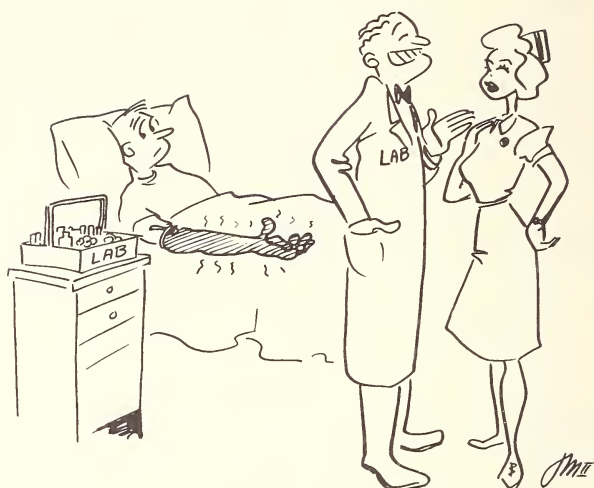


Figure 10. Laboratory personnel must act with intelligence and concern in obtaining specimens from patients.

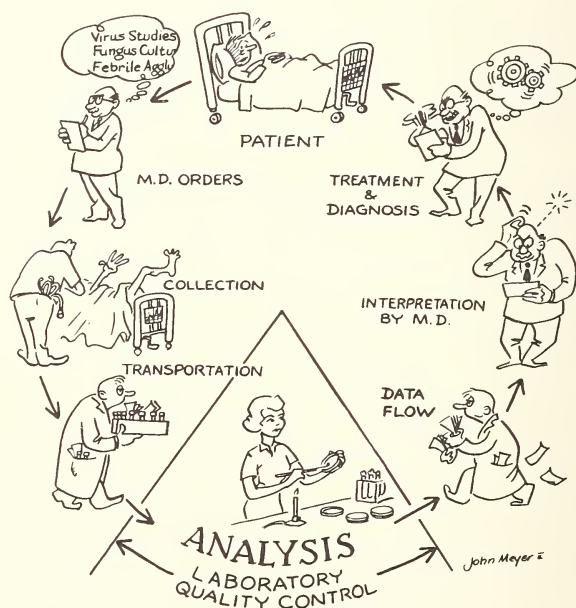


Figure 11. More errors occur during collection, transporting and data handling than within the laboratory since the introduction of quality control programs.

**b. Method and Personnel Interactions.**—Senior medical technologists who have achieved great manual manipulatory skills often resent and resist the introduction of new methods; especially instrumental methods which tend, in their eyes, to reduce the importance of their contributions. There is, therefore, an understandable reluctance by many clinical chemists to incur the displeasure of the technical staff by making method changes too frequently. Yet when analytical upgrading is not constantly taking place in a laboratory, the technological “shock” of later transitions may be overwhelming as suggested in Figure 12. Technological obsolescence can occur very rapidly today for both the clinical laboratory and its personnel.

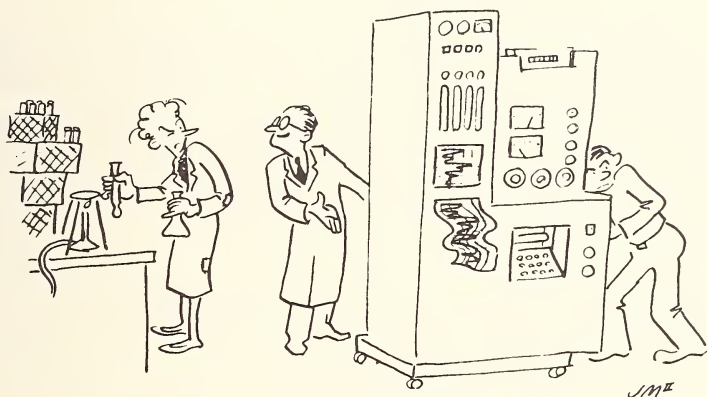


Figure 12. Analytical upgrading of methods and instruments must constantly take place or the “technological shock” of later transitions may be overwhelming.

Our own experience with new instrumental methods and revision of older manual methods since 1959 reveals (from Table 4) that only seven of the thirty-eight methods employed prior to 1960 have not changed. Perhaps more importantly, however, has been the chemistry division personnel changes which have brought in the senior scientific chemical and engineering skills needed to permit the orderly incorporation and heavy dependence upon instruments. Thus each year clinical chemistry has become more and more a team effort. No individual can any longer bring to the task all of the developmental, technical, chemical, engineering and management skills required. One needs only scan the “1971 Annual Reviews of Clinical Chemistry” by Kingsley in this past April’s issue of *Analytical Chemistry* [19] to appreciate how impossible a job one faces if he decides to “go-it-alone”.

c. **Communications with physicians and others.** — It is very important that the chemist communicate actively and effectively with the physician who must order the clinical chemistry tests. "Laboratory Notes" represent our annual effort each June to publish for the medical staff a comprehensive listing of tests available, the normal values, analytical precision, special information and precautions in sampling as well as the method reference [20]. As noted in its introduction, more detailed instructions are available in the "Nursing Procedure Book" or "Rapid Rolodex Files" which are available at each of the eighteen nursing stations throughout the hospital where physicians initiate written orders on their patients. Alternatively, laboratory phones are always manned with personnel who can answer routine queries. To further assure that tests will be available when needed, a senior clinical chemist is always "on-call" by phone, and physicians and laboratory staff are urged to contact him directly. Therefore the physician's dilemma shown in Figure 13 can often be resolved by a few simple tests which are or can be easily obtained—if the physician knows of their availability or by conversation with the on-call chemist, orders them. By many informal routes, as well as those described, effective communications with physicians must be accomplished.



Figure 13. The physicians dilemma in diagnosis of coma can often be resolved by a few relatively simple chemistry tests. Moral—when perplexed, talk with your friendly clinical chemist.



When special tests are not performed within our laboratory, we also take the responsibility after consultation with the physician to send the specimen to an outside laboratory. Appendage B lists those 32 types of tests which were referred out in 1970 (a total of 1,080 tests). Because of frequent conversations with those requesting these outside services, the rationale and priorities for adding new tests to our laboratory are more easily determined. Since outside referral tests are generally fairly complex, the average cost to the patient is \$25/test *versus* our average charge for chemistry tests of approximately \$3/test.

### 5. Chemistry Costs vs. Charges—The Hospitals' Dilemma

The annual costs for operating chemistry services at Hartford Hospital presently are calculated to be about \$1,000,000. Since the test volume is approaching 500,000, the basic cost is approximately \$2/test. However, due to the fact that we, like most hospitals, cost account the entire laboratory as a unit and do not split out chemistry costs, the charge to the patient is nearer \$3/test in order to defray the entire pathology department costs.

With the advent of automation, venture capital and aggressive laboratory management, many private clinical laboratories are now providing limited chemistry services (*i.e.*, screening profiles) at lower charges than do hospitals. This is due primarily to the fact that hospital laboratories have the extra costs of (1) teaching programs (2) autopsy expenses (3) round the clock fast-response services for emergencies and (4) costly services like microchemistry which averages not \$2/test but closer to \$10 while charges average \$5/test. To date neither hospital administrators nor pathology laboratory directors have been willing to say that outpatients are exempt from these costly inpatient hospital service charges. The larger private non-hospital clinical laboratories have, therefore, had much more latitude to lower charges and gain in volume.

As a result all across the nation we are seeing the rapid emergence of two types of clinical chemistry laboratories. One, typically a unit within a hospital dedicated primarily to inpatient services, fast-response and 24 hour emergency testing and the second, generally private and detached from the medical center, providing high volume out-patient testing for physicians' offices with heavy dependency upon automated technology mainly the Technicon SMA 12/60 units.

Ultimately regional chemistry laboratories incorporating more of the flexible management and the efficiency of the private enterprises, yet providing all of the services of the present hospital laboratories, will



evolve. The most logical way to assure both the quality and quantity of services under the direction of skilled laboratory scientists is to have these laboratories be an integral part of the medical community they support. These regional service organizations will be of great scientific complexity and to maintain their excellence must be centers of education, of original research and of innovative instrument developmental activities.

## B. SPECIAL CONSIDERATIONS IN CLINICAL ENZYMOLOGY

“It is, I think, difficult to exaggerate the importance to biology, and I venture to say to chemistry no less, of extended studies of enzymes and their action.”

Hopkins [21]

As a specific illustration of the rapid technological changes which now characterized clinical chemistry laboratories and their responsiveness to biomedical research events, let me review with you the phenomenal growth of clinical enzymology in the past fifteen years. In 1950 the Hartford Hospital Clinical Chemistry Laboratory, in common with most hospital laboratories, offered only four enzyme assays: amylase and lipase to help detect pancreatic disease, acid phosphatase as an aid in diagnosis of malignant prostatic tumors and alkaline phosphatase for the diagnosis of diseases of bone, of liver or of neoplastic origin. By 1955 the total volume of clinical enzyme tests by these four assays was approximately 6,000 or 9 percent of the chemistry tests performed in that year. Now 15 years later in 1970-71, 12 separate enzyme assays are offered routinely and another 5 are available when required for special needs.<sup>1</sup> Last year the total volume of enzyme assays was 91,000 or 21 percent of the present total chemistry workload. Figure 14 shows how rapidly some of the newer enzymes used in diagnosing heart disease have caught on in the last few years.

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<sup>1</sup> With the advent of isoenzyme assays and a multitude of newer serum, urine and RBC enzyme assays it would be possible to list 50 or more separate determinations that might be performed. For example, one might assay the red blood cells activity of delta aminolevulinic acid dehydrase activity since this enzyme has shown good correlations with blood lead levels, however, the direct measurement of blood lead is already available.

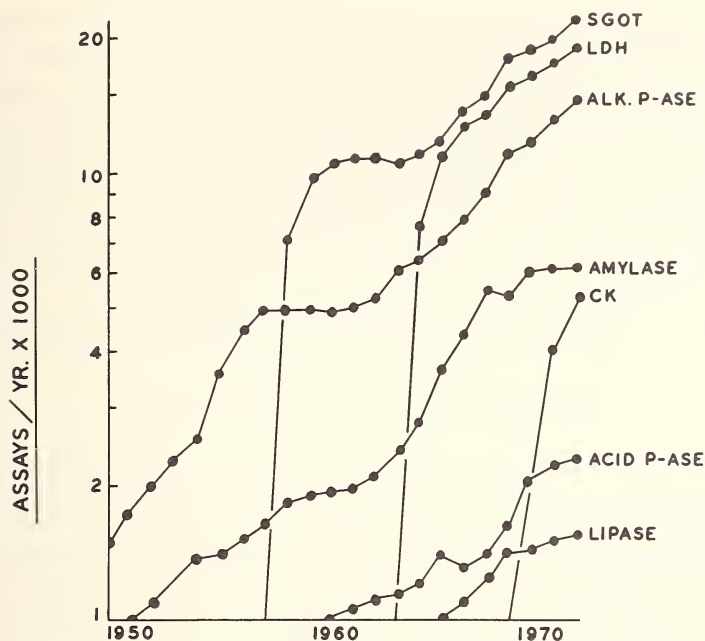


Figure 14. The yearly growth in clinical enzymology is shown. Note the marked increases in SGOT, SGPT, LDH and CK used in following suspected or diagnosed patients with heart attacks.

### 1. Transaminase—Introduction and Research

The great impetus to clinical enzymology began in 1954-55 when serum glutamic oxaloacetic transaminase (SGOT)<sup>2</sup> was introduced as an aid in diagnosing patients suspected of having an acute myocardial infarction (heart attack). At first clinicians were amazed and openly skeptical that the very minute amount of this enzymatic protein circulating in serum could offer help as claimed in the differential diagnosis of chest pain. However, in patient after patient and in study after study conducted between 1955 and 1957 this SGOT assay gave information which correlated very well with the history and the highly esteemed traditional electrocardiographic evidence of infarction of the heart. Within months doubts were replaced with enthusiastic acceptance in every institution in

<sup>2</sup> Enzyme classification (EC) No. 2.6.1.1 - L-Aspartic 2-oxo-glutarate aminotransferase. The popular name of this enzyme in the United States is serum glutamic oxaloacetic transaminase (SGOT) or "the heart transaminase." Some publications use the term aspartic aminotransferase.

which the test became available. Today one seriously questions the diagnosis of acute myocardial infarction if enzyme changes are absent. Figure 14 also shows that the subsequent introduction of lactic dehydrogenase (LDH)<sup>3</sup> and creatine kinase (CK)<sup>4</sup> has also resulted in heavy utilization of these two enzymes in patients with suspected myocardial infarctions.

The original research investigations and published reports on the transaminase enzymes can be traced back to 1937 to the work on pigeon breast muscle by two Russian workers, Braunstein and Kritzmanon [22]. A few years later in 1940, the Americans Cohen and Hekhuis [23] showed the presence of two distinct transaminase enzymes in rat tissues. The glutamic oxaloacetic transaminase (GOT) gave activities as follows:

heart  $\gg$  muscle > brain > liver > kidney.

In contrast, the glutamic pyruvate transaminase (GPT)<sup>5</sup> activity was highest in liver but very low in heart. In 1954-55 three New York biomedical investigators, LaDue, Wroblewski and Karmen [24], tested to see if these differences in organ activity were actually reflected in the serum after injury to an organ in cancer patients. Fortunately they noted marked elevations in the SGOT enzyme of several post-operative patients suffering from transmural myocardial infarctions. The diagnostic significance of these observations was not missed and their findings were communicated quickly in widely read publications [25]. By 1957 the value of the SGOT assays in the clinical practice of cardiology was amply confirmed and had become firmly established in numerous medical centers all over the world. The pressure to supply SGOT assays routinely in all hospitals receiving patients suffering from heart attacks was tremendous. However, by the early 1960's SGOT assays were generally available and as one direct consequence of the rapid growth in clinical enzymology the need for chemists in clinical laboratories increased considerably.

## *2. SGOT Methodological Considerations*

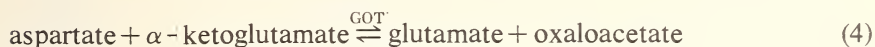
The original assays of serum SGOT were performed by indirect techniques requiring laborious paper chromatographic separations of the

<sup>3</sup> EC No. 1.1.1.27, L-lactate: NAD oxidoreductase.

<sup>4</sup> EC No. 2.7.3.2, ATP: creatine phosphotransferase.

<sup>5</sup> EC No. 2.6.1.2, L-Alanine 2-oxo-glutarate aminotransferase. The popular name of this enzyme in the United States is serum glutamic pyruvate transaminase (SGPT) or "the liver transaminase." Some publications use the term alanine aminotransferase.

products. Karmen [26] soon introduced a unique and relatively simple ultraviolet assay for SGOT determinations which permitted clinical laboratories throughout the world to measure this important enzyme in serum or any tissue with great ease. By coupling a product of the GOT reaction, oxaloacetate, to the coenzyme dependent malic dehydrogenase (MDH) (see Equation 4 and 5) the continuous detection of GOT activity was possible. The rate of change at  $A_{340}$  nm under carefully controlled conditions is directly proportional to GOT activity.



The spectral absorbance curves of reduced NAD and NAD which are associated with these redox changes are shown in Figure 15. The continuous ultraviolet spectrophotometric technique for measuring the activity of coenzyme dependent enzymes (or any components of these systems) had

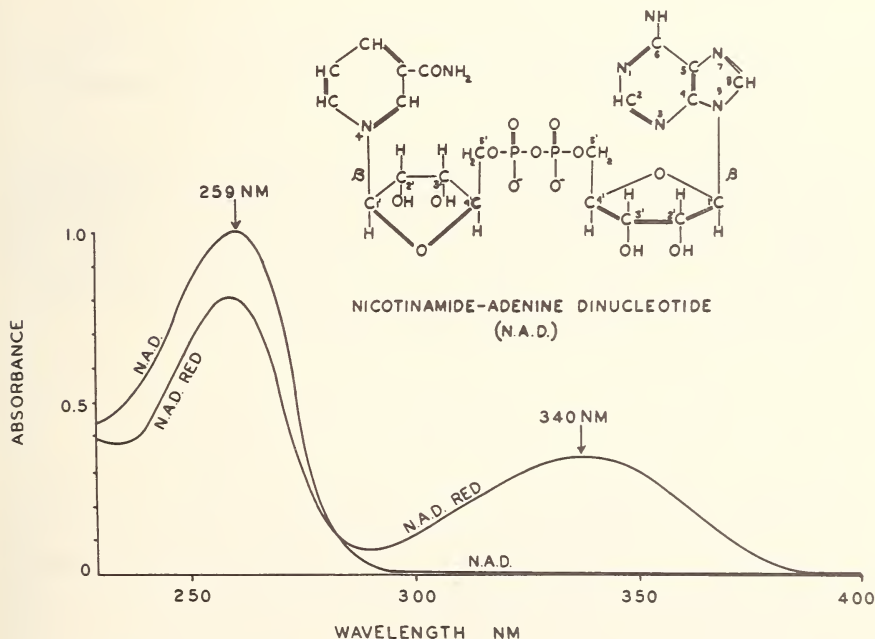


Figure 15. The spectral absorbance curves of NAD and reduced NAD. The change in absorbance at 340 nm permits the activity of many enzymes to be measured continuously (*e.g.* transaminase).

been described and developed extensively by Warburg and Christian [27] in the mid 1930's. Since there are nearly one-hundred coenzyme dependent systems known to biochemistry today, the importance of thoroughly characterizing these coenzyme materials and having them available in ultra-high purity is obvious. Fortunately the need for coenzyme standard reference materials has been recognized by those in charge of the NBS Standard Reference Materials (SRMs) Program and I am told that work is to begin on NAD in the Analytical Chemistry Division at NBS.

Over the last decade many people have suggested modifications in Karmen's malic dehydrogenase coenzyme linked system for GOT assays as follows:

- a. changes in concentrations of reduced NAD, of aspartate, and of  $\alpha$ -ketoglutarate.
- b. higher activity and purity of MDH enzyme to speed the oxaloacetate conversion and exclude GOT contamination, respectively.
- c. inclusion of LDH to destroy pyruvate endogenous to serum (avoids preincubation).
- d. closer control of kinetic and instrumental sources of variability (*e.g.*, temperature).
- e. changes in the buffer from phosphate to 2-amino-2-hydroxy-methyl-1,3 propandiol (Tris).

These modifications were generally aimed at reducing the causes of variability in GOT results seen in both intra and inter-laboratory comparisons. Although alternative chemical systems based upon single or double-point sampling colorimetric techniques have been introduced, none has received the widespread national and international study and endorsement of Karmen's continuous spectrophotometric procedure.

### *3. The Clinical Utility of Enzyme Measurements*

The time-activity pattern of serum glutamic-oxaloacetic transaminase (SGOT) changes during the course of an uncomplicated myocardial infarction is given in Figure 16. About six to twelve hours after onset of chest pain, abnormal serum activity can be detected for the first time. Subsequently, a peak activity four to five times the upper limit of normal (4N-5N) is reached near 36 hours followed by return to normal levels by the fourth day. Note that the serum glutamic-pyruvic transaminase (SGPT) remains within or close to normal limits throughout this period.



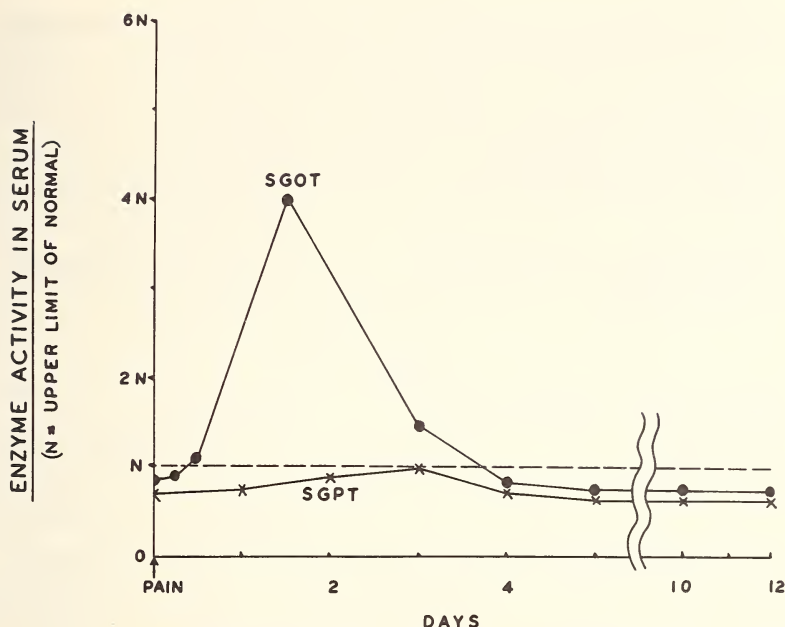


Figure 16. The daily activity pattern of SGOT and SGPT following the onset of an acute myocardial infarction.

To more fully appreciate the dynamic shifts between intracellular and extracellular fluids which these serum enzyme patterns of activity reflect, the article by Posen entitled, "Turnover of Circulating Enzymes" should be reviewed [28].

As enumerated in Table 7, there are many other biological changes which also occur during damage to cardiac muscle which are reflected in serum. The time-activity patterns of some of the other helpful serum enzyme changes in comparison to the SGOT are given in Figure 17. The very short-lived rapid elevation of the isocitric dehydrogenase<sup>6</sup> (IDC) of cardiac origin is seen within one to three hours after infarction. This heart isoenzyme of IDC is very heat labile and has usually disappeared well within the first twenty-four hours. The serum IDC isoenzyme of liver origin is heat stable and will often rise during the later course of an infarction of the heart if there is a complication causing liver damage. This IDC assay [29] is rarely used today and is little needed since the introduction of creatine kinase.

<sup>6</sup> EC No. 1.1.1.42 L-isocitrate: NADP oxidoreductase.

Table 7. Biochemical changes reflecting cardiac muscle damage.

Enzymes	
SGOTransaminase	Aldolase
Lactic dehydrogenase	Phosphohexosisomerase
LDH fraction one	Malic dehydrogenase
Hydroxybutyric dehydrogenase	Isocitric dehydrogenase
Creatine kinase	(heat labile fraction)
Other	
Serum copper (ceruloplasmin)	Complement
Serum iron	C-reactive protein
Serum nickel	Pyruvate
Erythrocyte sedimentation rate	Lactate
White blood count	

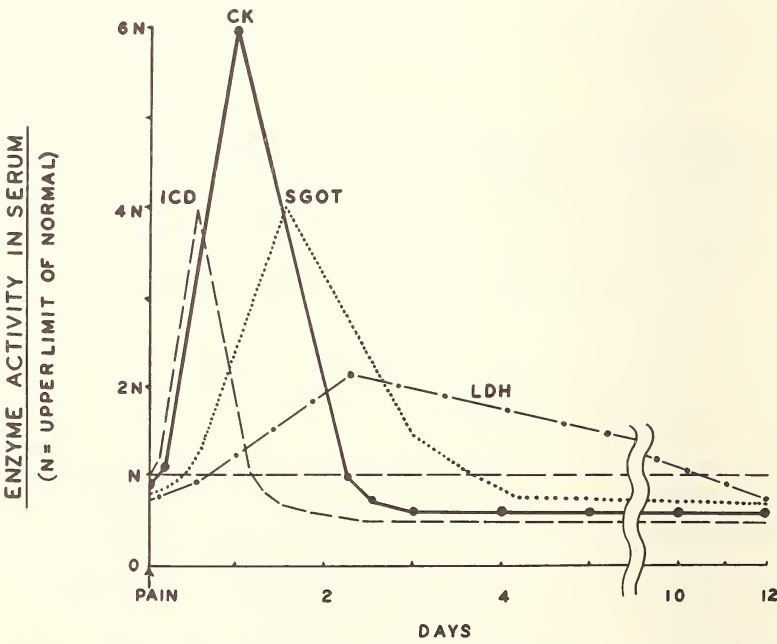


Figure 17. The daily activity pattern of other serum enzymes in contrast to the SGOT during an acute myocardial infarction.

The serum creatine kinase (CK) enzyme has a time-activity course which is very similar to that of SGOT except that the rise, peak, and return to normal all occur a few hours earlier. Although the assay is much more complex than SGOT, serum CK assays offer the distinct advantage of greater clinical specificity as damage to other body tissues, especially liver, does not release this enzyme into serum. Only skeletal muscle injury causes alterations and this is usually easily excluded.

Serum total lactic dehydrogenase (LDH) and its component isoenzyme fraction found predominantly in heart (LDH<sub>1</sub>) have also been utilized extensively to diagnose myocardial infarctions. The more sensitive and more clinically specific LDH<sub>1</sub>, as well as the total LDH, does not return to normal levels for 10 to 14 days making the assay very useful in patients admitted to the hospital several days after the onset of their infarction. Between 95 and 100 percent of patients proven to have an acute myocardial infarction at autopsy have been shown to have these SGOT, LDH or CK changes when studied properly.

Alterations from the typical time-activity patterns seen in myocardial infarction often suggests that the patient has another cause underlying the chest pain. The time-activity patterns shown in Figure 18 represent the enzyme changes seen during the hospital course of a 50-year-old woman admitted in shock to Hartford Hospital with a history of chest pain. The tentative diagnosis of myocardial infarction was supported by electrocardiographic abnormalities compatible with, but not diagnostic of, acute heart damage. Both transaminase enzymes were markedly elevated and the exceedingly high abnormal SGPT level suggested a tissue source other than or in addition to heart muscle. The high serum amylase obtained on the next day as a result of this high SGPT level lead to the correct diagnosis of acute pancreatitis which was later found to be secondary to cholecystitis with cholelithiasis.

There would be little difficulty to present numerous similar enzyme time-activity patterns involving transaminase and many other serum enzymes offered daily in the laboratory which have helped physicians to reach a correct diagnosis. Without the enzymes many of these diagnoses would have been missed or not made at all until a subsequent admission when the disease process was far more advanced. Although clinical enzymology is already a very meaningful area to clinical medicine, it is obvious that there lie ahead opportunities for innovative approaches with enzymes to the rapid diagnosis of common diseases seen in daily practice as well as the solution to some maladies which have plagued man for centuries.

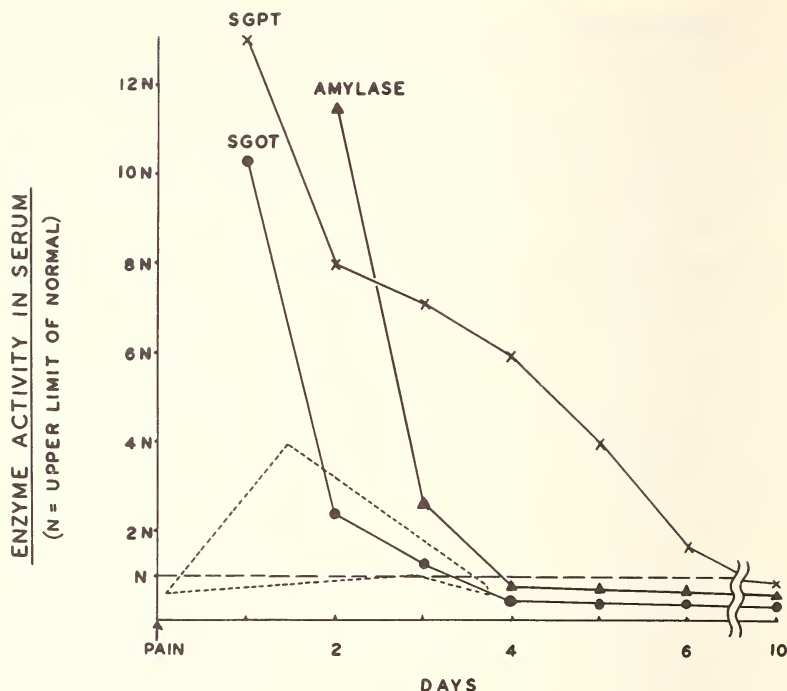


Figure 18. The daily activity pattern of serum enzymes during an acute bout of pancreatitis which was clinically thought to be a heart attack.

### III. Current Analytical Problems and Opportunities

"The history of science shows that even during the phase of her progress in which she devotes herself to improving the accuracy of the numerical measurement of the qualities with which she has long been familiar, she is preparing the materials for the subjugation of new regions, which would have remained unknown if she had been contented with the rough methods of her early pioneers. I might bring forward instances gathered from every branch of science showing how the labour of careful measurement has been rewarded by the discovery of new fields of research, and by the development of new ideas."

Maxwell [30]

Several analytical problems, and therefore opportunities, found in the health areas will be discussed in this section. The order and weighting of

the following is strictly my own and again reflects a deep concern for meaningful quantitative measurements within medicine.

### A. STANDARDIZATION

Until very recently only a few medical researchers and clinical chemists have come into this field with a background of analytical chemistry. Therefore, the skepticism about chemical measurements which is the traditional hallmark of the analytical chemist has not necessarily always characterized medical research or clinical chemistry.

Standardization efforts in service laboratories have increased in recent years partly as a result of more chemists with analytical training entering medical laboratories, but mainly due to the surveys which have clearly demonstrated the need for better performance. Congress officially recognized this national problem by passage of the Interstate Clinical Laboratory Improvement Act of 1967 (CLIA 1967). The government proficiency testing under this CLIA '67 and also the Medicare inspections by state personnel are increasing the emphasis on standards.

The need for improved clinical standards and standardization procedures received special attention by analytical chemists in 1968 in a paper entitled, "Standard Reference Materials as Reviewed by a Laboratory Supervisor—A Status Report" published in *Analytical Chemistry* [31] by the Subcommittee on Reference Materials, Committee on Analytical Chemistry, Division of Chemistry and Chemical Technology, National Academy of Sciences—National Research Council. Today, only three years later, in 1971 the availability of high purity certified organic and inorganic standards has improved considerably as shown in Table 8 taken from Dr. Meinke's recent article, "Standard Reference Materials for Clinical Measurements" [32]. I recommend the reading of this article by anyone concerned with quality measurements in the medical laboratory.

In the protein area, Dr. Theodore Peters and a Protein Subcommittee of the Standards Committee, American Association of Clinical Chemists, have already proposed specifications for a primary protein standard [33]. Standard material, bovine serum albumin, meeting these specifications has now been achieved by several manufacturers and the National Committee for Clinical Laboratory Standards (NCCLS) has accepted these specifications as the first "Tentative Standard" in clinical chemistry. I should point out that there is a great opportunity for anyone interested in



Table 8. Standard reference materials for clinical measurements.<sup>a</sup>

SRM No.	Name	Purity (%)	Property certified	Amount (g)	Date issued
40h	Sodium oxalate	99.95	Reductometric standard	60	April 24, 1969
83c	Arsenic trioxide	99.99	Reductometric standard	75	Feb. 6, 1962
84h	Acid potassium phthalate	99.993	Acidimetric standard	60	July 9, 1969
136c	Potassium dichromate	99.98	Oxidation standard	60	March 24, 1970
186Ic	Potassium dihydrogen phosphate	99.9	pH	30	July 29, 1966
186IIc	Disodium hydrogen phosphate	99.9	pH	30	Sept. 1, 1970
350	Benzoic acid	99.98	Acidimetric standard	30	April 15, 1958
911	Cholesterol	99.4	Identity and purity	0.5	Oct. 20, 1967
912	Urea	99.7	Identity and purity	25	Sept. 24, 1968
913	Uric acid	99.7	Identity and purity	10	Sept. 24, 1968
914	Creatinine	99.8	Identity and purity	10	Sept. 24, 1968
915	Calcium carbonate	99.9	Identity and purity	20	March 4, 1969
916	Bilirubin	99	Identity and purity	0.1	March 10, 1971
917	D-Glucose	99.9	Identity and purity	25	Nov. 18, 1970
918	Potassium chloride	99.9	Identity and purity	20	Jan. 22, 1971
922	tris(Hydroxymethyl)amino-methane	99.9	pH	25	May 1, 1971
923	tris(Hydroxymethyl)amino-methane hydrochloride	99.7	pH	35	May 1, 1971
930	Glass filters for spectrophotometry		Absorbance	3 filters	Feb. 24, 1971
1571	Orchard leaves		Major and trace constituents	75	Jan. 28, 1971
2201	NaCl	99.9	pNa pCl	120	April 15, 1971
2202	KCl	99.9	pK pCl	160	May 1, 1971

<sup>a</sup> Taken from Meinke, W. W., Standard Reference Materials for Clinical Measurements, *Anal. Chem.*, 43, 28A (1971).

protein chemistry and its standardization problems to join Dr. Peters in developing standards for human serum albumin and various globulins.

### *1. pH Standardization—A Model to Avoid Chaos*

The importance of NBS-SRMs to the clinical field is easily demonstrated by comparing the uniformity in standardization which exists in whole blood pH measurements. Because of the early leadership by NBS personnel over the years in providing a centralized authoritative base for measurements involving hydrogen ion activity with various electrodes, the chaos in standardization which exists in many other areas of complex measurements (*e.g.*, proteins-enzymes) has been almost totally avoided. Essentially all manufacturers and users of blood pH meters now employ the same standards to evaluate the performance of their instruments. The introduction in 1961 by Bower, Paabo and Bates [34] of the extremely useful 0.04 *M* physiologic dual salt phosphate buffer (SRMs Numbers 186 Ib-KH<sub>2</sub>PO<sub>4</sub> and 186 IIb-Na<sub>2</sub>HPO<sub>4</sub>) giving  $7.381 \pm 0.001$  pH units at 37 °C was very timely and is today the central reference point for clinical blood pH measurements in this country.

Dr. Richard Durst tells me of yet another addition to the NBS buffers intended for use in standardizing clinical pH measurements on whole blood—this one a Tris-Tris HCl buffer matrixed in physiological concentrations of sodium and potassium to more closely simulate the ionic matrix of whole blood. Certainly as we move to use ion selective electrodes other than for pH measurements, I would expect that the reference standards would contain more than the one element under study in order to more fully parallel the physiological specimens. This standardization of ion selective electrodes is a critical area requiring much more intensive investigation by chemists before widespread clinical applications occur. The principal problems are the limits of specificity and the little understood binding of the ion of interest to various proteins.

### *2. Standardization Problems Peculiar to Enzymology*

It is common knowledge among enzymologists that there are absolutely no accepted benchmarks to help one standardize enzyme activity measurements. However, the need for standardization in enzyme measure-

ments has been apparent for many years. For example, in 1956 when an International Enzyme Commission of ten distinguished enzymologists<sup>7</sup> was appointed by the International Union of Biochemistry, they were charged "To consider the classification and nomenclature of enzymes and coenzymes, their units of activity and *standard methods of assay* (italics mine), together with the symbols used in the description of enzyme kinetics" [35]. It is interesting to note that the final Committee recommendations in 1961 and those endorsed by International Union of Biochemistry in 1964 deliberately avoided specific recommendations concerning standard methods of assay except to give general advice on the conditions of measurement. Despite this lack of enzyme method standardization, the IUB 1964 Enzyme Commission Recommendations have been exceedingly successful in standardizing symbols and the activity units.

However, the need for method standardization still exists and is particularly acute in clinical enzymology. I personally believe standardization must include not only the methods of assay, but also the stable human source enzyme reference materials which must validate the method. While some people fear that selection of standard methods for enzyme assays by national or international organizations will stagnate enzyme method development, I firmly believe that the very existence of a standard method of assay is required to unify methods research by giving an agreed-upon starting point of reference.

**a. Enzyme Standardization Activities.**— In the United States a Subcommittee on Enzymes of the Standard Committee of the American Association of Clinical Chemists (AACC) is developing recommendations for standardized methods to assay serum enzymes. At the same time there is a separate effort within the Subcommittee on Enzymes of the Area Committee in Clinical Chemistry-National Committee for Clinical Laboratory Standards (NCCLS), to develop reference enzyme materials of human origin. Both groups recognize that enzyme standardization is very much like the two faces on a coin; each face can be viewed separately but it is really not possible to detach one face from the other.

Thus, the AACC Committee on methods recognizes the need for suitable human source enzyme materials to adequately define the operational characteristics of the methods they choose. Conversely, the NCCLS Enzyme Subcommittee members cannot really choose the most likely human source of stable enzyme reference material without evaluating them with

<sup>7</sup> M. Dixon (England), E. F. Gale (England), S. P. Colowick (U.S.A.), A. L. Lehninger (U.S.A.), A. E. Braunstein (U.S.S.R.), W. A. Engelhardt (U.S.S.R.), K. Linderstrom-Land (Denmark), P. A. E. Desnuelle (France), F. Lynen (Germany), O. Hoffmann-Ostenhof (Austria).

validated methods. Hopefully within 1972, these joint efforts of AACC and NCCLS should result in almost simultaneous recommendations on enzyme methods and materials for at least one or more of the following: (1) alkaline phosphatase—human purified enzyme from placenta (2) aspartate aminotransferase—human purified enzyme from red blood cells (3) lactic dehydrogenase—human purified enzyme fraction LDH<sub>1</sub> from red blood cells and (4) prostatic acid phosphatase—human crude enzyme from prostate and/or semen.

There are several groups in Europe concerned with enzyme standardization. There was a national symposium in Italy in 1969 [36] and I have just received correspondence from Professor A. Burlina telling of another international symposium which is scheduled for November 27-28, 1971 in Conegliano Vento, Italy. Correspondence from Professor R. Keiding reports of the formation of a committee of the Scandinavian Association of Clinical Chemists concerned with increasing the precision and accuracy of clinical chemical enzyme assays.

In England a working party of the Association of Clinical Biochemists composed of well known clinical enzymologists<sup>8</sup> who provide overlap through individual memberships with the Association of Clinical Pathologists and The Royal College of Pathologists, has issued its first report concerning standardization of the alkaline phosphatase King-Armstrong Unit. This report gives a manual and an automated reference method for serum assay of alkaline phosphatase complete with detailed notes [37]. A second report concerning the serum GOT assay is due soon. There has recently appeared a report from Germany which bears further discussion, but first it would be remiss not to note that the Standards Committee of the International Federation of Chemists has appointed an Expert Panel<sup>9</sup> to bring together recommendations on standardization of enzyme assays and reference material.

The German Society of Clinical Chemistry through its Commission for Enzyme Diagnostics and Standardization has published recommendations on eight serum assays<sup>10</sup> under the title "Standardization of Methods for the Estimation of Enzyme Activity in Biological Fluids" [38]. This German Committee recognized the major importance of "optimized conditions" established by appropriate experimental evidence in specifically recommending the need for the following conditions: (1) The continuous monitoring of enzyme kinetics. (2) The reaction rate should be constant

<sup>8</sup> D. W. Moss — Chairman, D. N. Baron, P. G. Walker, and J. H. Wilkinson.

<sup>9</sup> G. N. Bowers, Jr., Chairman (United States), H. U. Bergmeyer (W. Germany) and D. V. Moss (United Kingdom).

<sup>10</sup> Lactic dehydrogenase, lactic dehydrogenase-fraction one, glutamic dehydrogenase, glutamic oxaloacetate transaminase, glutamic pyruvate transaminase, creatine kinase, alkaline phosphatase and leucine arylamidase.



during the whole period of observation. (3) The kinds and concentrations of substrates, cofactors, activators and buffers should result in optimal reaction conditions. This relates to substances as well as to concentrations. (4) The reagents should be free of inhibitors. (5) The measurement temperature should be 25 °C; measurements at 25 °C are easier to standardize than at 30 °C; a temperature of 25 °C can be kept constant and conforms with the international temperature for physio-chemical data.

This German Committee has made a significant contribution in being the first national clinical chemistry society to publish specific recommendations on standard assays. The difficulties to be overcome in reaching other national and subsequently international agreements on standardized enzyme methods and/or standardized reference enzyme materials should not be underestimated. However, reducing the tremendous chaos which presently exists in the absence of such agreements will undoubtedly make the task somewhat more rewarding.

I would now like to discuss in greater detail some of the problems associated with developing enzyme materials and measurement systems in the light of some of my personal experiences with alkaline phosphatase over the last decade.

**b. Standardized Reference Enzyme Materials—Experience with Alkaline Phosphatase.**—The need to develop a human alkaline phosphatase standard reference enzyme material is of greater importance today than people not working in this area would believe. As an example, in 1967 we published on the “Variability of Analytical Results in a Survey Reference Sample Related to the Use of a Non-human Serum Alkaline Phosphatase” [39]. This paper documented the marked differences seen between the human pooled serum enzymes and other materials containing alkaline phosphatase enzymes from unknown sources. Studies were performed on human serum pool material, an unknown survey material, four commercial control materials, and four crude enzymes from (a) chicken intestinal mucosa, (b) bovine intestinal mucosa, (c) hog kidney and (d) the *E. Coli* enzyme of dog feces. Each source of enzyme showed markedly different kinetic characteristics when tested in relationship to heat stability, stability to alkaline denaturation, and inhibition by phosphate or phenylalanine. In my judgement, none was similar to the serum enzyme(s) to permit its use without warning users of the differences.

I am always amazed to see the simplistic assumptions and frank compromises people glibly accept in using reference materials containing enzymes. After spending months to perfect a method or instrument to perform assays on the serum of patients, they frequently seek to validate the



enterprise by using non-human enzyme materials of which little is known beyond the name on the bottle.

Several queries will help one to evaluate reference materials containing enzymes. **First**, what is the base protein of the pool? Not all preparations of pools start with native human serum but instead many use reprocessed outdated blood bank plasma or even animal plasma or serum as the protein matrix. **Second**, what is the species and tissue source as well as the method of preparation of the enzymes added to the base pool prior to lyophilization? What kinetic differences are introduced by non-human source enzymes *versus* the enzyme(s) in human serum? **Third**, how has the labelled activity value been assayed? If the exact details of the assay methods are not made available it is impossible to verify by independent means any given activity value. Often activity is measured by one method and factors used to calculate activity to be expected by another. And, **fourth**, what is the stability of the materials in the dry or frozen state and then after reconstitution or thawing?

In general, experience over the years suggests that relatively few commercial manufacturers of quality control or reference materials containing enzymes have the factual data to give one an answer to all these questions. Happily, one or two companies have recognized that open and free scientific communication and cooperation is as important as momentary proprietary advantages. These companies have published the sources of added enzymes and their assay methods. Some have even supported efforts to develop human source reference enzyme materials *via* the NCCLS Committee. It is fortunate that mechanisms like NCCLS committees now exist which can obtain a consensus among users, manufacturers, and government on standardization problems in the U.S.A.

Since at this time neither the added enzyme materials nor the assay method can possibly be considered to be "standard" (implying a broad consensus), it is ridiculous to suggest as has been done frequently in the past that some commercial products are "**enzyme standards in serum.**" Moreover, it is almost impossible with the present lack of agreement in standardization in clinical enzymology for any manufacturer or individual to certify the enzyme activity of a serum without providing complete information about the four questions asked above. Yet, each day thousands upon thousands of patients have their serum alkaline phosphatase activity determined on instruments which have been calibrated solely by accepting labelled values.

From many conversations with manufacturers of quality control materials I am sure that most commercial concerns would welcome standardization efforts in clinical enzymology by authoritative competent

committees. The manufacturers recognize better than anyone else the utter chaos which exists at present. In Figure 19, Dr. Meyer has tried to capture the frustrations of the analyst who relies too heavily on the label values of enzyme activity from various preparations of control materials each containing a different source of enzyme. Needless to say, help from persons who can bring order to clinical enzymology will be welcomed.

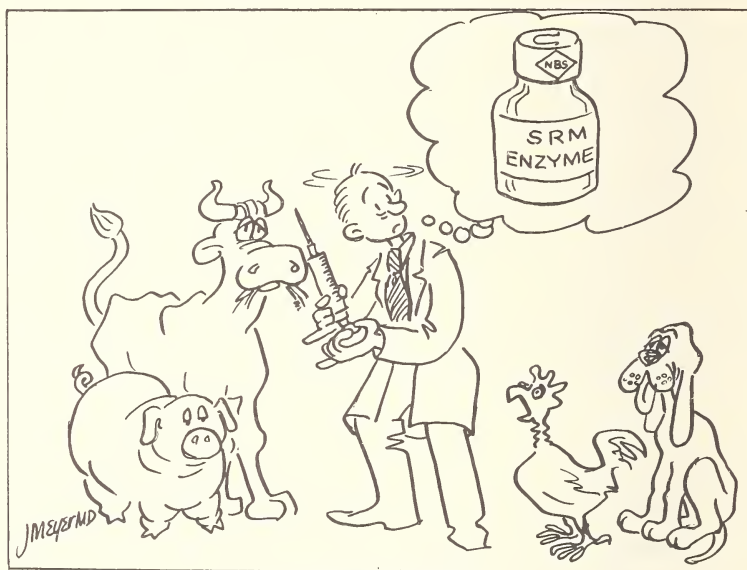


Figure 19. SRMs for enzymes are as yet only a dream. The barnyard is full of non-human sources of enzymes but they may be troublesome substitutes.

**c. Standardized Enzyme Methods—Experience with Alkaline Phosphatase.**—Table 9 lists just a few of the numerous methods that have been used to measure serum alkaline phosphatase over the last 40 to 50 years. It is obvious that trying to choose any one method arbitrarily as a standard assay method would be difficult. However, the conditions set forth by the IUB Enzyme Committee in 1964 would immediately eliminate a considerable number of these methods since they are not optimal. The German Standardization Committee has specifically recommended continuous monitoring of enzyme kinetics and the experience in our laboratory would endorse this choice over sampling techniques.

In 1963 my biochemist-colleague, Dr. Robert B. McComb, and I were convinced by preliminary investigations that a continuous spectrophotometric assay for serum alkaline phosphatase could be perfected.

Table 9. Serum alkaline phosphatase methods.

Author	Substrate	Buffer-pH	Time-temperature	Product measured-comments
Robison (1922)	Glucose- $\text{PO}_4$	- - -	3 days - 37°	$\text{PO}_4$
Martland, Hausman and Robison (1924)	Glucose- $\text{PO}_4$	- - - 8.4	2 days - 37°	$\text{PO}_4$
Kay (1930)	B-glycerol- $\text{PO}_4$	(serum), 7.4	2 days - 37°	$\text{PO}_4$
Jenner and Kay (1932)	B-glycerol- $\text{PO}_4$	veronal, 9.4	3 hours - 37°	$\text{PO}_4$ , higher pH shortened incubation time
Bodansky (1933)	B-glycerol- $\text{PO}_4$	veronal, 8.6	1 hour - 37°	$\text{PO}_4$
King and Armstrong (1934-58)	phenyl- $\text{PO}_4$	carbonate-bicarbonate, 9-10	15-30 min	many modifications in the way to measure the released phenol
Ohmori (1937)	p-nitrophenyl- $\text{PO}_4$	- - -	- - -	self-indicating product, p-nitrophenol
Shinawora, Jones and Reinhardt (1942)	B-glycerol- $\text{PO}_4$	veronal, 9.3	1 hour - 37°	$\text{PO}_4$ , higher pH gave more hydrolysis than Bodansky
Huggins and Taladay (1945)	phenolphthalein-di $\text{PO}_4$	glycine, 9.7	15 min - 37°	self-indicating product with added NaOH
Bessey, Lowry and Brock (1946)	p-nitrophenyl- $\text{PO}_4$	glycine, 10.3	15 min - 37°	reduced amounts of serum with self-indicating product on addition NaOH
Hofstee (1954)	carboxyphenyl- $\text{PO}_4$	phosphatase, 4-10	15 min - 37°	self-indicating product

Table 9. Serum alkaline phosphatase methods (continued).

Author	Substrate	Buffer-pH	Time-temperature	Product measured-comments
Seligman (1957)	$\beta$ -naphthyl- $\text{PO}_4$	Veronal, 9.1	15 min - 37°	measured released $\beta$ -naphthyl
Marsh (1959)	phenyl- $\text{PO}_4$	carbonate-bicarbonate, 10.0	15 min - 37°	King-Armstrong method adopted to AutoAnalyzer®
Moss (1960)	$\beta$ -naphthyl- $\text{PO}_4$	carbonate-bicarbonate, 10.0	10 min - 37°	fluorimetry, $\lambda_{\text{ex}} = 335$ nm $\lambda_{\text{em}} = 455$ very sensitive
Garren and Leventhal (1960)	p-nitrophenyl- $\text{PO}_4$	Tris, 10.0	- - -	continuous type assay with self-indicating product
Fisher and Siebert (1961)	phosphoenolpyruvate	diethanolamine-8.5	20 min - 37°	coupled with LDH to give changes in NAD at 340 nm
Morgenstern, Kessler, Auerback, Flor and Klein (1965)	p-nitrophenyl- $\text{PO}_4$	2A2M1P, 10.2	15 min - 37°	AutoAnalyzer® sampling method with self-indicating substrate
Bowers and McComb (1966)	p-nitrophenyl- $\text{PO}_4$	2A2M1P, 10.15	5 min - 30°	continuous kinetic assay at 404 nm
Coleman (1966)	thymolphthalein mono- $\text{PO}_4$	2A2M1P, 10.15	10 min - 25°	sampling type method with color developed with NaOH 590 nm
Bowers, Kelley and McComb (1967)	phenyl- $\text{PO}_4$ o-carboxyphenyl- $\text{PO}_4$ $\alpha$ -naphthyl- $\text{PO}_4$ $\beta$ -naphthyl- $\text{PO}_4$	2A2M1P, 10.15	10 min - 25°	kinetic method self-indicating products at 288, 298, 334, 347, 555, 595 and 476 nm, respectively

Table 9. Serum alkaline phosphatase methods (continued)

Author	Substrate	Buffer-pH	Time-temperature	Product measured-comments
Rick and Hausamen (1967)	phenolphthalein mono- $\text{PO}_4$	diethanolamine- 9.8	6 min - 25°	spectrophotometric continuous at 400 nm kinetic assay increased molecular activity over 2A2MIP
	phenolphthalein di- $\text{PO}_4$			
	thymolphthalein mono- $\text{PO}_4$ and $\alpha$ -methylfluorescein- $\text{PO}_4$			
Neumann and Vreedendal (1967)	p-nitrophenyl- $\text{PO}_4$	$\text{NH}_4\text{OH}-\text{NH}_4\text{Cl}$ , 9.8	- - -	self-indicating substrate, buffer claimed to be less inactivating of enzyme and have good buffer capacity
	p-nitrophenyl- $\text{PO}_4$			
Nath and Gosh (1967)	p-methylphenyl- $\text{PO}_4$ and 16 methyl 9 fluoroprednisolone- $\text{PO}_4$	- - -	- - -	- - -
	4 methylumbelliferyl- $\text{PO}_4$			
Cornish, Neale and Posen (1970)	Naphthol As-BI	carbonate- bicarbonate, 9.2	8 min - 37°	automated fluorometric assay
Guilbault and Coworkers (1971)	p-nitrophenyl $\text{PO}_4$	2A2MIP, 9.8	5 min - 25°	fluorimetry = $\lambda_{\text{ex}} = 405$ $\lambda_{\text{em}} = 515$ nm
McComb and Bowers (1971)	p-nitrophenyl $\text{PO}_4$	ethyl-amino- ethanol, 10.15	5 min - 25°	increased molecular activity over 2A2MIP



As shown in the spectral absorbance curves given in Figure 20, the rate of enzymatic activity of alkaline phosphatases is easily monitored from the changing absorbance near 400 nm as the colorless substrate p-nitrophenyl phosphate is hydrolyzed at the alkaline pH of 10.15 to the highly colored (self-indicating) product, p-nitrophenol. This substrate was first introduced by Ohmori in 1937 [40]. It has been utilized in the well-known sampling type assay for serum alkaline phosphatase introduced by Bessey, Lowry and Brock in 1946 [41]. Lowry and coworkers in a prior paper had remarked about the increased activity found in propanolamine buffers [42]. These buffers were little used until Garren and Leventhal employed tris in a study of the alkaline phosphatase of *E. Coli* in 1967 [43].

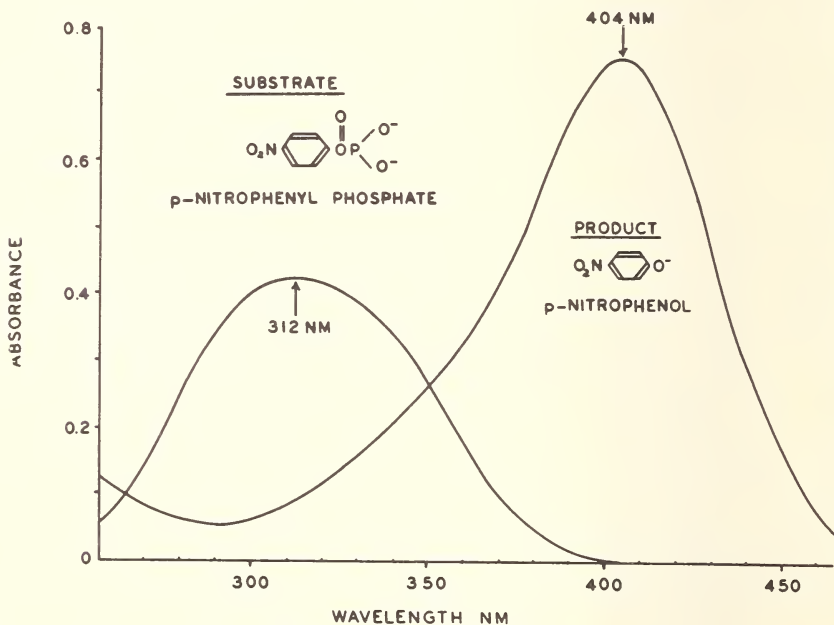


Figure 20. The spectral absorbance curves of one alkaline phosphatase colorless substrate and self-indicating product which permits direct continuous spectrophotometric assays.

Subsequently, Amador, Zimmerman and Wacker employed a continuous type kinetic assay to measure the low levels of alkaline phosphatase found in human urine using the buffer 2-amino-2-methyl-1-propanol [44]. These authors did not apply the continuous type assay to serum choosing instead the traditional Bessey, Lowry and Brock procedure. During the August 1964 Annual Meeting of the American Association of Clinical

Chemists our paper and one read by Klein [45] described almost identical experiences in measuring serum alkaline phosphatase in 0.75 mol/liter of 2-amino-2-methyl-1-propanol (2A2M1P) at pH 10.15 at 30 °C. This buffer accentuated the molecular activity 4 to 5 times over the glycine buffer used by Bessey, *et al.*, as was shown in our subsequent publication [46].

After extensive experience over several years in both routine and experimental work it was believed that this continuous alkaline phosphatase assay system had been sufficiently described and tested to qualify as a candidate for a standard enzyme method. It successfully met the exacting conditions set forth by the IUB Commission of Enzymes in the 1964 report as well as the conditions of the recent German publication. However, in 1967 Hausman, *et al.*, [47] described another propanolamine buffer, diethanolamine (DEA), which gave twice the molecular activity with p-nitrophenyl phosphate. Subsequently, this "DEA" method has received the German Commission's endorsement. Continued examination of numerous other buffers in our laboratory has revealed yet another propanolamine buffer, ethyl-amino-ethanol (EAE), with superior buffering capacity and 50 percent higher molecular activity than DEA as shown in Figure 21 [48].

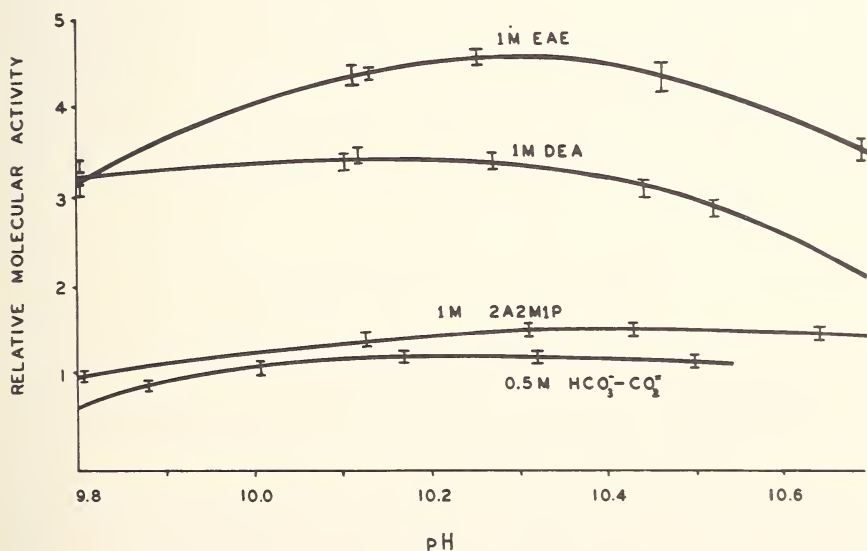


Figure 21. Serum alkaline phosphatase activity as a function of pH and differing buffers. 2A2M1P = 2-amino-2-methyl-1-propanol, DEA = diethanolamine, and EAE is ethylaminoethanol.

This experience with several different buffers, all quite satisfactory, illustrates how difficult it will be to select a standard assay method even when all conditions are in agreement with the recommendations of learned bodies. There is agreement at present, however, that continuous monitoring of enzyme kinetic activity is a prime consideration. The details of other conditions, *i.e.*, buffer type, must be resolved by further intensive bench work and an open review of all data by the various official committees. It is my present belief that standard enzyme methods after fulfilling the criteria previously listed must also be tested extensively with the stable human source enzyme materials which are now currently under development. In addition it will be necessary to test sera from numerous patients with diseases associated with the major isoenzyme changes (bone, liver, intestine and placental) before making final decision on any standard method for serum alkaline phosphatase assay or for that matter any other serum enzyme.

The job will be difficult since there are no absolute goals—only empirical observation and many strong, sometimes conflicting, opinions. Even tentative choices of expert panels and other official committees will at best be arbitrary and subject to much debate. However, once national and subsequently international agreement has been reached, critical scientific examinations of new systems *versus* the established standard enzyme assay method are sure to create a continued pressure towards revision with methods proven to be superior. We need not fear ossifications with standardized assay methods for once a goal is set it is the very nature of man that there will be many who will attempt to surpass it (the goal of a few years ago of a four-minute mile is now the three-minute fifty-second mile!).

#### B. REFEREE METHOD DEVELOPMENT

“Believe those who seek the truth; distrust those who claim to have found it.”

Andre Gide [49]

For many years the American Association of Clinical Chemists has published a series of volumes entitled, “Standard Methods of Clinical Chemistry” [50] which has attempted to identify methods which have performed well in a submitter’s laboratory and have been proven by test to be satisfactory in several checker’s laboratories. The editorial board of

senior clinical chemists and the editor of each volume review all data to assure that the methods published are constantly updated to reflect the daily working experience in clinical laboratories. Although due concern is given for precision and accuracy, the primary aim is to describe in detail methods which will reliably handle the daily workload within a time span which is realistic for service laboratories.

There has been a growing recognition of the need for methods which are designed **primarily** to give excellent **precision** and **low systematic bias** without the limitations imposed by time, efficiency and technical skill. These **referee methods** would seek to establish the "true" content of a serum constituent more closely than is possible with present routine daily production methods. A referee method would be utilized, therefore, to establish the bias of the more commonly employed methods. Once accepted across the country, referee methods could also prove extremely useful in uniformly setting the label value for the serum control materials required to calibrate automated instruments which do not accept primary standard solutions.

Obviously the precision required of a referee method cannot be set by routine daily quality control data which have given the "allowable error" of Tonks [51] or the subjectively chosen "medically significant values" of Barnett [52]. I believe the objective criteria set by Cotlove, *et al.*, as the "tolerable analytical variability" [53] and by Young, *et al.*, in the "average intra-individual biological variation" [54] must be utilized to help set the overall precision plus accuracy goals for these referee methods. Table 10 gives the "average intra-individual biological variation" from Young's paper in comparison to the long-term precision data<sup>11</sup> observed in the Clinical Chemistry Laboratory at Hartford Hospital for the period October 1969 to the end of September 1970. I would certainly expect that the relatively short-term batch to batch analytical precision of a properly functioning referee method to be  $\pm 1$  percent RSD, or less; except for the serum enzymes, cholesterol and creatinine.

The accuracy to be expected of referee methods is yet to be established. While accuracy cannot exceed the purity of the standard (see Table 8), ideally it should approach this limit subject only to the uncertainty of the random analytical variability. Since to my knowledge there is no factual information yet available on systematic bias of extremely precise referee type methods, the accuracy goals listed in Table 10 are admittedly subjective and arbitrary. However, our rather intensive and now extensive work in attempting to determine the systematic bias associated with the mea-

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<sup>11</sup> Based on 80 to 120 days of a "blind" serum pool at multiple levels.



Table 10. Referee method goals.

Test (mean—units)	Average intraindividual biological variation <sup>a</sup> % RSD	Long-term laboratory variation <sup>b</sup> % RSD	GOALS		
			Preci- sion % RSD	Accuracy ±% from 100%	Uncertainty <sup>c</sup> ±% from 100%
Sodium (144 mmol/l)	1.4	1.2	0.5	0.5	1.5
Potassium (108 mmol/l)	4.6	2.3	1.0	0.5	2.5
Chloride (25 mmol/l)	2.7	2.1	1.0	0.5	2.5
Carbon dioxide (25 mmol/l)	5.3	4.0	1.0	0.5	2.5
Calcium (2.5 mmol/l)	1.6	1.3	0.5	0.5	1.5
Magnesium (0.85 mmol/l)	2.4	2.4	1.0	0.5	2.5
Phosphate (3.8 mmol/l)	9.9	4.3	1.0	1.0	3.0
Total protein (7.4 g/dl)	2.2	1.5	1.0	1.0	3.0 0
Albumin (4.9 g/dl)	3.0	4.5	1.0	1.0	3.0
Uric Acid (6.1 g/dl)	8.8	2.9	1.0	1.0	3.0
Urea nitrogen (18 mg/dl)	13.5	3.2	1.0	1.0	3.0
Glucose (94 mg/dl)	6.5	3.0	1.0	1.0	3.5
Cholesterol (204 mg/dl)	4.2	4.5	2	2	6
Creatinine (1.1 mg/dl)	4.4	5.3	2	1	5
SGOT transaminase (6.4 U/l)	15 —	11 17	3 3	—	—
SGPT transaminase (5 U/l)	—	17	3	—	—
Lactic dehydrogenase (126 U/l)	19	8	3	—	—
Alk. phosphatase (45 U/l)	5.8	8	3	—	—

<sup>a</sup> See: Young, D. S., Harris, E. K., and Cotlove, E., Biological and Analytic Components of Variation in Long-Term Studies of Serum Constituents in Normal Subjects: IV Results of a Study Designed to Eliminate Long-Term Analysis Deviations, *Clin. Chem.*, 17, 403 (1971).

<sup>b</sup> Hartford Hospital, '69-70. See also Appendage A.

<sup>c</sup> These represent the opinion of the author on what is achievable for Referee Methods only. The uncertainty was calculated as (2 × precision + accuracy).



surement of total serum calcium by atomic absorption spectrophotometry (AAS), suggests to me that it is presently unrealistic to expect accuracy to exceed  $100 \pm 0.5$  percent [55]. Yet to suggest that inaccuracy exceed  $100 \pm 1$  percent except for enzymes and certain complex unstable biological substances would be to expect too little of our measuring capabilities.

With the availability of certified high purity standards from NBS as SRMs (Table 8) many chemists have asked, and quite properly so, how the NBS standard would help to improve the daily measurements on patients' samples. While it is obvious that SRMs in solution can be used to calibrate instruments or can be directly incorporated into the method, it is much less apparent to many that the very existence of the "certified" SRM, in and of itself, establishes a new, higher level of purity for clinical standards than ever before. For example, Witter and coworkers in 1965 demonstrated (and again in 1970) conclusively that cholesterol standards available on the market were mixtures of several compounds [56, 57]. Furthermore, even after following the recommended purification procedures, special storage conditions must be maintained if deterioration is to be prevented. Presently to assure against deterioration in cholesterol certified at the 99.4 percent level of purity, the NBS-SRM 911 cholesterol (plus at least one commercial supplier of cholesterol standards) package and store their highly purified materials under inert gases. Thus the specifications and analytical work behind the certification of an NBS-SRM effectively act to upgrade the standards sold commercially.

The second answer as to how an NBS-SRM will affect the accuracy of clinical measurements involves the development of these precise and accurate methods, these **referee methods**. An NBS-SRM or a comparable high purity material must be available as the logical starting point and central focus of any referee method. Without such a high purity certified standard even comparative method evaluations become rather meaningless. The high purity chemical standards are used in all calibrations of instruments both in simple solutions and in as nearly an identical matrix as possible to the matrix of the unknown. The method is then tied to multiple standards closely bracketing the level of the unknown. By proper statistical design and experimentation, the random error components from each procedural step are evaluated and reduced.

As an example of the way the accuracy of a referee method must be evaluated, I will again draw on our recent experience in trying to determine the systematic bias associated with measuring calcium by AAS. The same highly skilled analytical chemist<sup>12</sup> performed all the steps of six dif-

<sup>12</sup> Mr. John Pybus, Visiting Clinical Chemist—Hartford Hospital, June 1969 to June 1971. (Senior Scientific Officer, on leave, Auckland Hospital, Auckland, New Zealand).

ferent AAS methods and two minor variations for measuring total calcium in serum. The Pybus, Feldman and Bowers method [55] which is now being proposed as a referee method<sup>13</sup> gave an identical result (within  $\pm 0.7$  percent except for modification No. 8) to all the five other AAS methods as shown in Table 11. Perhaps this simply means that all AAS methods have a similar systematic bias! However, as shown in Table 12, an entirely different chemical isolation and separation route, that of oxalate precipitation with monitoring of supernatant losses of calcium, again gave an identical answer. It is important to note that a solution of NBS-SRM 915, calcium standard, went through each and every step of these methods. Our conclusion about the method was that it "appears to have a systematic bias of less than 1 percent."

Further, very recent experience in eight laboratories seeking to evaluate this proposed referee method for total serum calcium by AAS *versus* measurements by isotope dilution mass spectrometry (IDMS) at NBS suggests that the systematic bias is between 0.5 and 1.0 percent; however, the work on serum comparisons between AAS and IDMS has not progressed far enough at this time (July 1971) to suggest a closer figure.

The present studies of the NBS-Clinical Interaction Plan involving NBS personnel and several clinical laboratory scientists has clearly shown that precise and accurate measurements can be made in hospital and other clinical laboratories *when sufficient time and skilled personnel are committed to the task*. Unfortunately, all too few physicians demand and actively support the need for accuracy in clinical work which reports from the NIH group have shown are necessary [53, 54]. Most clinical judgements of a laboratory are based upon the repeatability of a split specimen or in a crude comparison of one or two results sent to two laboratories which may even use completely different methods. The cost of a determination seems to be far more important to many than any questions concerning the analytical credence. In many institutions today economic, not chemistry or service considerations seem to be the only criteria for making decisions. There is little or no concern over standards, proper standardization practices or meaningful quantitative measurements.

When we fully recognize the superb ability of our body cells to react to only one specific chemical configuration while apparently rejecting the influence of thousands of other chemicals in the fluids bathing our tissues, I am sure that the ultimate analytical goal for chemical measurements in

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<sup>13</sup> Joint NBS-Clinical Chemistry Interaction Plan.

Table 11. Comparison of six methods, showing the effect of various diluents on the results of Ca determination.

Column	1	2	3	4	5	6	7	8	9
Diluent <sup>a</sup>	La 10 <sup>a</sup>	La 10 <sup>a</sup>	La 10 <sup>a</sup>	La 10 <sup>a</sup>	La 18 <sup>a</sup>	EDTA 10 g/l	Sr 14 <sup>a</sup>	Sr 14 <sup>a</sup>	Means
Acid	50 mmol HCl/liter	50 mmol HCl/liter	50 mmol HClO <sub>4</sub> /liter	50 mmol HClO <sub>4</sub> /liter	50 HClO <sub>4</sub> / TCA	None	100 mmol HClO <sub>4</sub> /liter	50 mmol HCl/liter	—
Dilution	1:50 A/B <sup>b</sup>	1:50 A	1:50 A/B	1:50 A	1:20 A	1:50 A	1:50 A	1:50 A	—
Reference	[55]	—	—	—	[58]	[59]	[60]	—	—
Serum A	2.03 <sub>5</sub>	2.05	2.04	2.01 <sub>5</sub>	2.05	2.04	2.05	1.97	2.04
B	2.18	2.19	2.18 <sub>5</sub>	2.16 <sub>5</sub>	2.19 <sub>5</sub>	2.16 <sub>5</sub>	2.18 <sub>5</sub>	2.10 <sub>5</sub>	2.18
C	1.84	1.84	1.83 <sub>5</sub>	1.87 <sub>5</sub>	1.85 <sub>5</sub>	1.82	1.85	1.76 <sub>5</sub>	1.84
D	2.46 <sub>5</sub>	2.46 <sub>5</sub>	2.46	2.45	2.50	2.45 <sub>5</sub>	2.45 <sub>5</sub>	2.40	2.46 <sub>5</sub>
E	2.62 <sub>5</sub>	2.61	2.60	2.58 <sub>5</sub>	2.62 <sub>5</sub>	2.57	2.61 <sub>5</sub>	2.52 <sub>5</sub>	2.60 <sub>5</sub>
F	2.16 <sub>5</sub>	2.16 <sub>5</sub>	2.19 <sub>5</sub>	2.18	2.18	2.19 <sub>5</sub>	2.16 <sub>5</sub>	2.13	2.17 <sub>5</sub>
Mean	2.22	2.22	2.22	2.20 <sub>5</sub>	2.23 <sub>5</sub>	2.21	2.22	2.15	2.22 <sup>c</sup>
Percent change	—	—	—	-0.7%	+0.7%	-0.5%	—	-3.2%	—

<sup>a</sup> mmol/liter, except noted.<sup>b</sup> A = calcium by single channel alone. A/B = Ca/Sr ratio to an internal reference using dual channel.<sup>c</sup> Column (8) mean omitted.

Table 12. Ca in serum as measured by oxalate precipitation and the proposed method.

	Serum calcium, mmol/liter			
	Oxalate precipitation			Present method <sup>a</sup>
	Ca in ppt	Ca in supernatant fluid	Total	
Serum A	1.99	0.03	2.02	2.03 <sub>5</sub>
B	2.14	0.03 <sub>5</sub>	2.17 <sub>5</sub>	2.18
C	1.80 <sub>5</sub>	0.03	1.83 <sub>5</sub>	1.84
D	2.44	0.03 <sub>5</sub>	2.47 <sub>5</sub>	2.46 <sub>5</sub>
E	2.58 <sub>5</sub>	0.03	2.61 <sub>5</sub>	2.62 <sub>5</sub>
F	2.17	0.03	2.20	2.16 <sub>5</sub>
Mean	2.19	0.03	2.22	2.22
Percent	98.7	1.3	100	100

<sup>a</sup> La, 10 mmol, in HCl, 50 mmol/liter.

medicine will require degrees of sensitivity, precision, specificity and accuracy as yet rarely practiced. There is indeed a place for meaningful chemical measurements in medicine!

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## APPENDAGE A

Volume and Precision<sup>a</sup> on Individual Tests  
(Hartford Hospital 1969-70)

Test	Volume	% RSD <sup>a</sup>
Automation Section		
Glucose	48,391	3.0
Urea	39,650	3.2
Osmolality	5,511	1.9
Creatinine	16,121	5.3
Sodium	36,176	1.2
Potassium	34,350	2.3
Chloride	27,540	2.1
Carbon dioxide	27,051	4.0
Uric acid	6,925	2.9
Phosphate	7,256	4.3
Bilirubins	11,345	20
Thymol	1,592	16
Ceph. Floc.	1,289	--
C. S. F. Protein	2,871	5.0
Acetone	496	--
Xylose	119	--
Amylase	4,684	12
B. S. P.	497	--
Sweat Test	52	--
Electrolyte Section		
pH	5,942	0.3
Micro pH	47	--
P <sub>O</sub> <sub>2</sub>	5,054	--
Micro bilirubin	704	--
Calcium	9,600	1.3
Magnesium	1,462	2.4
Lithium	2,382	1.2
Barbiturate	316	--
Salicylates	130	--
Bromide	21	--
Doriden	10	--
Calculi	214	--
Cu, Zn, Pb	90	--
Hemog. S.	110	--
Iron	1,187	9

## Appendage A (continued)

Test	Volume	% RSD <sup>a</sup>
Protein—Enzyme Section		
Alk. P—ase	12,965	8
Acid P—ase	2,030	3.5
Lipase	1,411	2.3
Cholinesterase	413(77)	6
SGOTransaminase	19,448	11
SGPTransaminase	18,193	17
Lactic dehydrogenase	17,687	8
Ceruloplasmin	31	— —
Serum electrophoresis	4,544	4.5
Hgb electrophoresis	189	— —
Urine electrophoresis	85	— —
CSF electrophoresis	78	— —
T. Protein	5,642	1.5
A/G by electrophoresis	4,448	— —

## Endocrine Section

5 HIAA	128	— —
Cortisol, plasma	1,178	5.2
Protein bound iodine	2,468	5.4
Chromatog. urine AA	135	— —
17—Ketosteroids	640	17
17—OH corticosteroids	585	30
Cholesterol	5,880	4.5
Triglycerides	4,461	8
Metanephrine	89	— —
VMA	10	— —
Creatine kinase	4,051	8

## Nights

Glucose	4,770
Urea	3,647
pH	4,827
Sodium	4,035
Potassium	4,419
CO <sub>2</sub>	2
Micro bilirubin	135
Acetone	321
Amylase	1,221

## Appendage A (continued)

## Nights (continued)

Test	Volume
Barbiturates	152
Salicylates	117
Doriden	— — —
Osmolality	16
CSF Protein	15
Calcium	1
PO <sub>4</sub>	1
(Irons)	889

## Microchemistry

Glucose	15
Urea	42
Osmolality	1
Sodium	103
Potassium	105
Chloride	97
Chemical micro	5
Bilirubin	— — —
Alk-Phos.	8
SGOT	5
SGPT	5
LDH	5
Total protein	11
A/G by electrophoresis	10
Gulactosemia	3
Acetone	1

<sup>a</sup> Based on 80 to 120 days of a 'blind' serum pool at multiple levels.



## APPENDAGE B

## Tests Referred to Outside Laboratories

(Hartford Hospital 1969-70)

Aldolase	Growth hormone
Aldosterone	Haptoglobin
Bile acids	Iodine excretion
B <sub>12</sub> assays	Luteinizing hormone
Butanol extractable iodine	Epinephrine-norepinephrine
Catecholamines	Mucopolysaccharides
Cortisol secretory rates	Oxalate
Cortisone, urinary free	Phospholipids
17-OH Corticosteroids	Pregnanediol
(by Porter-Silber)	Pregnanetriol
11% Hydroxysteroids	Renin
Estrogens, fractionated	Testosterone
Fecal fat	Thyroid stimulating hormone
Free fatty acids	Total lipids
Free thyroxin	Triiodothyronine
F.S.H. pituitary gonadotropins	
Folic acid	



## PANEL DISCUSSION

### Analytical Problems in Biomedical Research and Clinical Chemistry



#### CHAIRMAN

Robert S. Melville, Chief, Clinical and Applied Sciences Section, National Institute of General Medical Sciences, National Institutes of Health



#### PANEL

George G. Guilbault, Associate Professor of Chemistry, Louisiana State University, New Orleans, Louisiana

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Howard V. Malmstadt, Professor of Chemistry, University of Illinois, Urbana, Illinois

George N. Bowers, Jr., M.D., Director, Clinical Chemistry Laboratory, Hartford Hospital (Speaker)

**Fales**—There are really two kinds of analytical chemistry connected with this topic: biochemical and clinical. Biochemical analysis is essentially a precursor of clinical analysis so that measurements are usually first made in the biochemical research laboratory, and then when the techniques are worked out, the method goes to the clinical laboratory.

There are two misconceptions which may pop up when analytical chemists consider the area of clinical chemistry. One is that if the analytical chemist dreams up some kind of instrument that works well, but is expensive, that it is automatically out. In practice this does not really seem to be the case. If the instrument gives the right answer, clinical chemists and biochemists will accept it and make good use of it.

A second idea is that there must necessarily be good theory relating the measurement to the disease condition. Certainly, it's fine if one can do this but often all one has is the correlation itself. Perhaps the SGOT measurement falls in this category; much more needs to be known about why it works—but it does work and it is being widely used.

In clinical chemistry extraordinary specificity is required. A number of years ago it appeared that gas chromatography and thin-layer chromatography would provide the answers to many of the clinical chemist's problems, and it seemed that the methods would be used widely in the clinical laboratory. Unfortunately, because of their lack of specificity, the methods have never gotten off the ground in the average clinical environment. They definitely have utility today, but more to confirm results, and for research purposes than in the routine clinical laboratory. So a technique which sometimes looks very, very good indeed often is discarded because of lack of specificity. What is really needed in the clinical field is a system which is routine and, hopefully, mechanizable.

In contrast to situations in the clinical field, scientists in the purely biochemical areas are often confronted with the need for a one-shot analysis in order to solve a particular problem. In fact, several months might be spent gearing up to run a single analysis if the question is important enough. Furthermore, in biochemical analysis the scientist is trying to get the widest range of information possible from a given technique. Therefore he uses broad range methods such as nuclear magnetic resonance and mass spectrometry because they give such a complete picture of the total system. There is also more of a tendency to deal with ultra-sensitive methods in biochemical research as contrasted with clinical problems.

Sometimes in the clinical field there are special requirements; for example, in the field of drug analysis rather poor quantitation is satisfactory but there should be no false identifications. If a laboratory reports that a person has been taking morphine, this report must be made with a very high

degree of reliability since inaccurate information could damage a person's reputation or dictate improper treatment. For example, recently the District of Columbia contracted for drug analyses in the methadone program and the requirement was that there would be no more than one false positive in two hundred analyses! It seemed to me that it would be very difficult for any clinical laboratory to meet these specifications but in fact one laboratory has met the specification and been awarded the contract.

Another area that has special requirements is the emergency identification of drugs in overdose cases. Here the requirement is that the analysis should be very fast, *i.e.*, less than an hour, and there must be an absolutely positive identification of the drug, *i.e.*, not just a "barbiturate" but *what* barbiturate. Or: is it doriden? Quantitation is much less important.

One of the main requirements for such analyses, touched on by Dr. Bowers, is that there must be a very close association between the clinical chemist and the physician. Too many hospitals seem to have their clinical chemistry controlled by a very non-chemically-oriented pathologist. It must be realized that if a person is in the field of clinical chemistry, he is there primarily because he is interested in applying his chemical know-how to help sick people. Thus, he ought to be given an opportunity on occasion to observe the patient at first hand along with the doctor. In this way the clinical chemist would feel much more a part of the overall clinical picture. The more such cooperation can be arranged, the more reliable the advice of the clinical chemist will become, since he will be able to see at first hand how his measurements contribute to the overall clinical situation.

Regarding more exotic methodology, particularly techniques which might be in the near future, it seems to me that mass spectrometry may well become a widely used method in the clinical laboratory. After all, one of the fundamental properties of matter is mass; if one knows the mass of a molecule, one really knows quite a bit about it—including information as to whether it has one or two nitrogens, *etc.* The mass limit of modern mass spectrometers is only somewhere between 500-3000 mass units and right now it is necessary to volatilize samples. Someday we should be able to get around this requirement. It really is rather ludicrous to start with an ionic substance, derivatize it in order to vaporize it, then return it to an ionic form so that it can be analyzed in the mass spectrometer.

EPR will make an important contribution in the future. The "FRAT" system of the SYVA Corporation has the combination of the specificity found in enzyme systems combined with the sensitivity available from measurements of electron paramagnetic resonance. Admittedly, the technique is only in the early stages of development at the present time, and confirmation by other methods is still desirable.



Fourier transform NMR methods are coming into use and may help us to get rid of the slits and scanning systems in spectrometry. This is a way to increase sensitivity by a factor of ten or decrease measurement time by a factor of one hundred, but it requires the use of a computer. Another interesting area is radiative detection of NMR signals which, in special cases, could provide NMR with the sensitivity available in radioactivity. X-ray crystallographic analyses will surely be more widely used—the surface has just been scratched. It is important to find ways to obtain good x-ray data from smaller and smaller crystals. Along with this, we need to find new ways to fish small amounts of pure crystalline material out of biological systems without a lot of work-up.

Much more work must be done on separation methods in general. Present day methods must somehow be speeded up. Some sort of immunological-type detector is needed. If it is known what compound is to be measured, there ought to be a way to “sensitize” a detector specifically to that compound. Nothing like this has been done to my knowledge. There needs to be some way to get total elemental composition data on pure materials at the picogram and nanogram level; microwave discharge has been suggested as a way of doing this.

But all of these things will not come to much unless we can find a way to increase the interaction between the analytical chemist, the clinician and the biochemist. It's a team effort today. This means we, as analytical chemists, are going to have to publish with biochemists and physicians in the biochemical and clinical journals.

An additional thought comes to mind. In these days when many organic and physical chemists are out of work there might be some possibility for retraining interested individuals in the area of clinical chemistry. Perhaps some support from NSF or another organization is required. (Others pointed out that there are committees working on these problems to develop a specific plan and thus detailed discussion at this time would probably be premature.)

**Guilbault** — A few comments are called for on the status of teaching of clinical analytical chemists in the colleges and universities today. Many years ago almost all of the premedical students and the medical technicians went through a good, thorough quantitative analysis course. But today, many of them do not. The reason for this is as much the fault of us in analysis as anybody else. To a certain extent we have “turned off” the M.D.'s and the people interested in the biomedical types of things by our old-fashioned courses.

To reverse this trend, a number of places have revitalized their courses. For example, now at LSU there is a course strictly in clinical methods for the medically-oriented people, the Med. Tech's., the M.D.'s, *etc.* In this course they do not do many of the old classical experiments but do a lot of new things. For example, they do experiments in atomic emission and atomic absorption for sodium and potassium in blood and urine. They study electrochemical methods, using sodium and potassium electrodes, and even use, experimentally, the new urea electrode. These methods have the advantage of selectivity and will be used a lot in the future to measure activity rather than just concentration, since activity is a more important parameter in many clinical measurements.

Measurements using fluorescence, for example, are made for magnesium in blood. They do TLC separations for drug identification; they also use GLC in the same way. Also spectrophotometric methods are used for urea and glucose in blood. These are examples of methods commonly used in many clinical laboratories. This gives the medically oriented people a much better idea of what types of analyses they will be running into in the future. At least the M.D. that comes out of this course in the next few years will have some kind of idea about what types of clinical tests are being performed for him.

Comments are appropriate on four specific problems in this area of clinical measurements. The first problem, that of standards, was well described by Dr. Bowers. There are a number of good standards available today that can be used for ion-selective electrodes, blood pH measurements, cholesterol, *etc.*; as well as actual biomedical samples, such as orchard leaves for calcium, potassium, iron, sodium, *etc.* Hopefully we can expect many more from NBS; this is a terrific service.

A second area to be mentioned is that of accuracy. Just what is a normal range? In many tests it has been shown that a so-called normal range is three-fold or more greater than what it should be. So how do we better define these limits? Presumably one could do a better job of getting a more representative "normal" individual or a whole range of representative individuals. But just how do we do this to improve the accuracy of the determinations?

Thirdly, better methods of analysis are needed, particularly for many of the new enzyme tests. It is estimated that enzyme tests grow at the rate of about 30 percent per year, and for many of these, better tests are still needed. Many of them lack analytical acceptance. New fluorescent methods, ion-selective electrode methods, enzyme electrodes, electrochemical methods, and others should improve the enzyme procedures of the future.

Fourthly, technician error is something that many of us do not recognize. When working with a large hospital in terms of trying to get some idea as to the errors involved in a test, there are many small things that the technicians might do which would invalidate or introduce greater errors into a particular procedure. A definite advance can be made through the development of technician-proof or "goof-proof" methods which require a minimum of solution preparation and manipulation on the part of the technician. This is the philosophy behind the development of enzyme electrodes. These will be very simple to use just like a pH electrode with essentially no special preparation. There are now electrodes for several materials such as urea, D- and L-amino acids, asparagine, glutamine, phosphate ion, and glucose.

Another aspect that is being approached is the so-called solid surface monitoring system which would completely eliminate all solution preparation. The technique here is to develop small pads upon which quantitative measurements can be made. One has a series of bottles with these pads. On the pads are all the reagents necessary to perform a particular assay. All a technician would have to do is to take a pad out, put it on a strip, and add the solution, whether it's blood or urine, in order to get the appropriate test. The pad would then be put in the instrument to take a reading, after having standardized the instrument. This would reduce greatly the possibility for technician error.

In summary there appears to be a great opportunity for the analytical chemist in clinical chemistry. It is almost like the Gold Rush Days in California—today there is a wealth of important analytical problems in clinical chemistry that are just waiting for the analytical chemist to rush in and offer his expertise.

**Karger** — A number of months ago Drs. Melville and Purdy described the critical shortage that exists for trained people in clinical chemistry. It is interesting to note that in a time when so many fields, including some of the chemical fields, are having difficulties in placing students that the area of clinical chemistry has such a diversity of opportunities.

To help remedy this situation the Department of Chemistry and the College of Pharmacy at Northeastern University in Boston are proposing a Masters Degree program in clinical chemistry for evening school part-time students. Students would be accepted with a BS degree in chemistry, biology, pharmacy or medical technology. Clinical chemistry spans several fields and it is unlikely that most students would have all the requirements one would like them to have. Thus in addition to the BS degree in one of the above fields, it is expected students will have two



quarters of physical chemistry, two quarters of organic, one quarter of analytical chemistry, and two quarters of human physiology. If a student does not have some of these courses, he would be able to take them in the part-time evening BS program.

The program will include about 20 courses, 10 of which will be required including courses from both analytical chemistry and from the pharmacy school. Included are a course in separations dealing with chromatography and other techniques, a course in electrochemistry, and a course in optical methods; three courses in biochemistry, and two courses in clinical chemistry where analytical techniques as applied to clinical chemistry will be discussed. There will also be a course in radioisotope techniques and one in concepts in toxicology. There will also be an elective core which could include chemical instrumentation and computers in chemistry; also special topics in analytical and clinical chemistry. Some students also might be interested in taking business courses since they might hope eventually to manage clinical laboratories.

One of the problems is that of clinical practice (for example, in the case of retraining persons whose primary background is physics, engineering, *etc.*). It is hoped to establish a cooperative program with one of the local hospitals in which a person would actually get practical laboratory training and experience over a period of three months.

Leaving the educational role analytical chemistry departments can play in clinical analysis, let us next turn to the research side. It is felt that high-speed liquid chromatography will play an important role in clinical analysis in the future. One example of future potential is in the area of steroid analysis. If one uses gas chromatography for steroid analysis, one must first have a hydrolysis step to obtain the free steroids from the conjugates. Then one may have to form derivatives before the sample is introduced into the gas chromatograph. In high-speed ion-exchange liquid chromatography one can separate directly the conjugates leaving out the hydrolysis and derivatization steps. The key here is that in liquid chromatography one can come close to the biological system with the sample that is injected. Liquid chromatography could also be very important in drug analysis, for example for the separation and analysis of barbiturates. In forensic analysis also, liquid chromatography can be of great value.

If you ask the question "Why has gas chromatography not become a more powerful tool in clinical chemistry?" you can get several answers. Dr. Fales has brought up the lack of specificity although one can point to many cases where very high specificity exists, *e.g.*, in the pesticide field. The problem appears to be more that the people that work in the field find gas chromatography a little too sophisticated to use routinely in the clini-

cal laboratory at the present time. Breakdown also appears to be excessive in relation to other methods such as simple colorimetric analysis.

**Malmstadt** — Analytical research chemists have a responsibility to make a study of all of the basic processes that occur in scientific measurements. George Bowers pointed out that 91 percent of the measurements made in his laboratory involved some kind of optical or spectrophotometric measurement. As analytical chemists we should be interested in the types of transducers and basic processes inherent in encoding chemical information as electromagnetic radiation information. At the same time we should be very much interested in the basic processes involved and the methods used in instrumentation to decode this information. We should not just be content to take some instrument as it has been given to us in the laboratory and use it for making scientific measurements. But we should be aware of all the basic processes that are involved in encoding and decoding and reading out of the information.

George Bowers mentioned the use of atomic absorption and flame emission techniques as well as the introduction of atom fluorescence methods which are today being used quite often in the clinical laboratory. In spite of the fact that there are many instruments available, there are many chemical processes involved in the encoding of the chemical sample elemental composition in the form of electromagnetic radiation, which are poorly understood. For example, more must be known about the desolvation processes, the vaporization and atomization processes, *etc.*, in order to make atomic absorption and atomic fluorescent measurements much more reliable, even though they are producing quite satisfactory results at the present time.

In the area of atomic fluorescence methods there are some exciting possibilities for the future in the use of tunable dye lasers, which should significantly improve sensitivities. There are also interesting recent experiments in atomic fluorescence and atomic absorption measurements in working directly with the solid sample without preliminary preparation. And this, of course, is of great interest for the clinical laboratory so as to eliminate the elaborate treatment of solid samples.

Another area where there should be concern is in the mechanisms of reactions. Too much of the early work was done on a very empirical basis, and that is one of the reasons why the clinical laboratories were not as good in the early days as today when they are reaching for more basic approaches.

Although this morning a lot was said about the problems in solid-state research it should be pointed out that scientists in this field have done



such a tremendous job that their devices are completely revolutionizing the instrumentation used for analytical measurements. As analytical chemists we are just beginning to see the fruits of that new foundation of electronics where entirely new design concepts have resulted from microcircuits and other innovations of the solid-state scientists.

What significance would a study of this new electronics have for the analytical chemist? Whereas several years ago it would take considerable time to try out certain instrumentation ideas, now an idea can be tested in a day or two. Thus it's a whole new ball game! If one has an idea of something that would improve sensitivity or remove interference, instead of taking six months or a year to develop it, it can often be tried out in a few days. Obviously, this can greatly expedite the development of new instruments and will have a great impact in the area of clinical chemistry.

Much has been said this morning and this afternoon about interfacing between individuals, the interface between the analytical chemist and the physicist, the metallurgist, and the physician. These are major problems, but there is also another type of interface that is of increasing importance and that is the interface between the analytical chemist, the clinical chemist and the machine—*i.e.* the man-machine interface. In the past it has been important to interface with instruments to try to know how to get the most out of them. But that kind of interface is going to become trivial in relation to problems of the very near future. In the past, time scales of years were available to adjust to a new, essentially manually-controlled instrument. But now as new automated instruments and automated laboratories come in to being, a whole new set of problems evolve in the man-machine interface.

Certainly this will be the decade of **complete laboratory automation**. This is from the point of view not only of routine work but also of research work. It is now possible to consider automating research approaches. Thus one can not only automate an instrument but automate entire research projects so that information which previously had taken many years to obtain might be obtained in a month or so. In the medical laboratory there is the possibility of automating the interpretation of a complete series of automatically obtained clinical analyses.

For our summer program in Digital and Analog Electronics for Scientists at the University of Illinois there has been much interest from people in the biological and medical sciences. Often they come in quite poorly prepared in instrumentation in relation to the physicist and engineer, but after spending several weeks in the course they are able to see how some of the new concepts in electronics and instrumentation can help them solve some of their problems. Not that they can necessarily design

the circuits or instruments, but they do know how to communicate with the people who would help them out.

This year, of the 400-500 applications for our summer courses, only about 10 were analytical chemists. Now this either means that analytical chemists are very well prepared in the new instrumentation and are ready for the new era of automation in the laboratory, or else they don't think it's important. Earlier in the day it was pointed out that analytical chemistry was a core discipline for analysis. It should be stated here that unless analytical chemists really learn about the new approaches to instrumentation and automation in the next few years, analytical chemistry will not be the core discipline for analysis.

**Bowers** — The rate of growth in terms of new tests for the clinical laboratory has essentially precluded the laboratory from going into some of the more sophisticated instrumental methods such as gas chromatography; the clinical chemistry laboratory in most institutions has just been overwhelmed! For example there is often a mandate to get a new test going almost immediately or the test will be sent to an outside laboratory. These pressing demands have frequently been for tests which amounted to thousands of samples a year such as blood gases at any hour—day or night—in contrast to the few which would employ gas chromatography for one or two analyses/week, usually for steroids or the area of drug analysis. It takes a great deal more time from senior analytical people to develop gas chromatography methods than most persons realize. One just doesn't walk up to the instrument and start turning out valid answers.

As regards the "Gold Rush" mentioned earlier it should be pointed out that in the past the clinical chemistry laboratory has often covered in its charges losses incurred from other hospital operations. There is now more competition from private laboratories, and the hospital laboratory charges are today more nearly reflecting costs. When hospital chemistry activities just recover chemistry costs, then there will be little reason for non-chemists to control clinical chemistry laboratories and I would hope a good chemist would be placed in charge of these vital laboratories.

A person coming into a clinical laboratory with a Ph.D. in analytical chemistry should plan on spending a minimum of two years in a laboratory as a post-doctoral fellow. The problem of interfacing with medicine is a major one. You just don't go into a clinical laboratory and suddenly change something where there are already strongly entrenched attitudes fixed by years of medical tradition. There needs to be an apprenticeship

approach to learn how to make these changes in a positive way. It can be a very exciting time but one does need to become familiar with the clinical surroundings and the peculiarities of medicine. However, there are many laboratories today where even undergraduate laboratory skill in analytical chemistry can provide an important contribution. If a person is really going to contribute to clinical chemistry in a major way, it is my contention he should equip himself with the knowledge and training of a Ph.D. chemist or/and M.D.





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## CHAPTER 4

# ANALYTICAL PROBLEMS IN AGRICULTURAL SCIENCE

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This paper presents a review and analysis of the widespread applications of analytical chemistry and analytical instrumentation in the research and regulatory programs of the Agricultural Research Service of the Department of Agriculture. Analytical chemistry is one of the essential sciences needed in advancing the commodity and people-oriented programs which can achieve the national goals of the Department. Chemical analysis will be shown to play a vital role in assuring an adequate supply of farm and forest products and in assuring the consumer a better product while minimizing costs for processing and distribution. In people-oriented programs, such as consumer health, safety and pollution abatement, analytical methodology is the key procedure for providing the basic data to get action programs into motion.

Along with the far-ranging applications of analytical chemistry in agriculture come many unsolved analytical problems. In fact, few agricultural scientists will admit complete satisfaction with any of their current methodologies. Yet some problems are more important than others. An assessment of these problems is made including an analysis of their relative importance in conjunction with the urgent needs of agriculture. Possible ways of classifying or categorizing these problems will be suggested. For example, amino acid analysis touches almost every branch of agricultural science whereas pesticide analysis is more restricted but has a great many more complexities. For any given pesticide, methodologies may be needed for controlling its application and for measuring its residues in free and bound form, its metabolites, trace contaminants, and the inert ingredients in its formulation.

Other problems are discussed for which there appears no current solution. This will challenge the ingenuity and creativity of analytical chemists in the future.

Keywords: Agricultural pollution; agricultural products analysis; agricultural science; amino acid analysis; biomedical research; clinical chemistry; diagnostic chemistry; food analysis; pesticide residue analysis.

## I. Introduction

Agricultural research is an extremely diverse field of effort. Agricultural commodities include fiber crops, oilseeds, animal products, fruits and vegetables, cereal grains, forages, sugar crops, tobacco, and new crops. Research on these commodities is carried out for a variety of purposes such as protection from losses, efficient production and quality improvement, product development and processing, efficient marketing, improvement of human nutrition and consumer satisfaction, and elimination of environmental pollution from the production and processing of foods. This diversity of commodities and objectives presents us with an extremely wide range of analytical problems.

To facilitate the consideration of some of our current problems by experts in the various analytical competences, we have arranged the problems topically rather than by commodity or type of activity. The problems, therefore, are arranged under the following subjects: elements and water, proteins and related nitrogen compounds, lipids, fibrous constituents of plants, nutrients and their availability, seed and plant constituents, animal products, sensory characteristics of foods, microorganisms in foods, animal health, biologically active substances, agricultural pollutants, molecular properties of natural polymers, and automation and instrumentation.

## II. Elements and Water

Starting with the simplest problems, from the standpoint of chemical composition, we would like to mention four problems that involve individual chemical elements and two problems concerning determination of water.

Recent unprecedented FDA actions in removing from or keeping off the market swordfish found to contain more than 0.5 ppm of mercury has made the public suddenly aware of widespread mercury contamination. Fortunately methodology for determining mercury at trace levels had been developed so that it was feasible to arrange to expand greatly and quickly the monitoring of fish for mercury content. The method now in general use involves chemical oxidation and reduction steps before the mercury is measured as vapor by atomic absorption. A determination requires only a few minutes. Perhaps this procedure could be automated

at least for similar samples in which the level of mercury content does not vary more than 10-fold. Nonetheless, it would be desirable to have a method which could be used to continuously monitor the mercury content of a water supply or effluent stream, with the possibility of using the output as a feedback to automatically control, perhaps, the operation of a mercury decontamination device which requires periodic regeneration. Another useful development would be portable equipment which could be used in the field or on a boat. It would also be prudent to seek similar rapid, automated methods for monitoring other toxic elements such as lead, cadmium, and arsenic in streams and in foods.

Of these elements, lead especially concerns us because it is exhausted into the atmosphere from automobile engines. Lead-contaminated crops are certainly used for human and animal food and lead-contaminated air is used in many food drying processes. How can this growing contamination be monitored?

The amount of lead now in foods must be measured in parts per billion. The classic dithizone procedures have this sensitivity, but are extremely tedious and would be very expensive for routine analysis. The recently developed atomic absorption methods are simple and fast, but are not sensitive enough for the low concentrations of lead found in foodstuffs.

Fortunately the technological explosion in the electronics industry has provided the means to solve most of these problems. In polarography, for example, the recent development of techniques such as the pulse and the fast scan oscillographic methods now permit analysis for metals such as lead near the part per billion level.

The main problem areas now appear to be in the relative complexity of the techniques and the sophistication required of the analyst. Instruments designed for routine repetitive analysis with a minimum of operator attention would be useful. Some method is needed for the easy acquisition of permanent recording of rapid scan oscillographic analyses. This would permit later careful study and could be of importance in regulatory procedures.

Another element that concerns us is bromine. Methyl bromide gas is widely used as insecticidal fumigant on cereals and cereal products such as flours and meals, on nuts and nutmeats, on dried fruits, and on some fresh produce. Experimental treatment of grain is shown in Figure 1. Annual U.S. production of methyl bromide in recent years ranged from 103 to 179 million pounds. Depending on the nature of the product treated, the concentration and exposure period may need to be carefully controlled to avoid exceeding the allowable tolerance for bromide residue, which varies from 50 ppm for cereals and pulses to 125 ppm for dried



Figure 1. Fumigant gas being fed into a grain storage bin for insect control.

fruits to 200 ppm for nuts. In the case of wheat it has been found that all residual bromide is inorganic within a few days of fumigation. The method generally used for determining residual bromide is that developed by Shrader, Beshgetoor, and Stenger [1] or some modification of it. It involves oxidation of bromide to bromate and determining the bromate iodimetrically. Its principal drawback is that it is time consuming and not sufficiently sensitive for determining a few parts per million. Much faster and sensitive methods such as neutron activation and x-ray fluorescence have been successfully used, but these require expensive equipment not



usually available in analytical control laboratories. A more sensitive and preferably simpler procedure than the chemical one now in use is still needed for routine control analysis for bromide residues in foodstuffs.

Sulfur is of interest because the current emphasis on replacing natural protein with nonprotein nitrogen in ruminant diets necessitates an evaluation of sulfur requirements. This has not been a problem in the past because natural protein contains adequate sulfur. The current sulfur methods are not suitable, especially for feed and feces samples when sublimed sulfur has been added to the diets of ruminants. The recovery rates are low, the equipment needed is expensive, and the procedures are slow and tedious. An efficient, accurate, and inexpensive method of measurement is needed.

Water activity of foodstuffs is an important consideration because the stability of many dehydrated, formulated, and other types of foods to various degradative processes depends on both the temperature and the moisture content. The thermodynamic activity of water in such a product is measured by the relative humidity of the atmosphere or gas phase in equilibrium with it. The relation between the water content and the equilibrium relative humidity (ERH) at a constant temperature is called the water sorption isotherm. Because sorption isotherms for different types of food components (such as sugars, fats, proteins, and carbohydrates) are quite different, those for different food products depend upon the composition. The sorption isotherm of a food product also varies with temperature.

For food formulation and new food product development work, stability characteristics are best observed and correlated in terms of ERH. On the other hand, most process and product control of moisture is more conveniently done through measurement of water content. The sorption isotherms needed to relate the two are now seldom determined because there is no rapid convenient method of doing so. Present methods involve either a point-by-point measurement of water content of material which has equilibrated with an atmosphere of known relative humidity (RH) or an estimation of this value from the rate of change of water content as the material equilibrates. Needed is automated instrumentation for determining sorption isotherm data on a suitably prepared sample over the RH range of approximately 5 to 85 percent for a wide range of temperatures and in a period of an hour or less.

The need also exists for a rapid response method for control of product moisture content in continuous fast drying of heat-sensitive materials. The quality of a number of dried food or feed products is sensitive to the temperature-moisture content history of the material during the drying

process. An important example is the rapid, continuous drying of alfalfa to produce dehydrated meal, which is of value as an ingredient in mixed feeds for poultry because of its xanthophyll content (pigmenting power) as well as its protein content. The xanthophyll pigments undergo serious degradation under the conditions existing in a dehydrator and the degree of loss (which varies from 30 to 70 percent) is related most closely to moisture content of the product at the discharge end even though rear end oven temperature is held constant.

Because the leaf and stem portions dry at different rates, the moisture content of the product as it leaves the drier is not uniform. Extremely careful control of operating variables such as inlet temperature, speed of rotation, air velocity, rear end temperature, and feed rate is essential for minimizing damage to the leaf fraction while drying the stem fraction sufficiently for good stability and handling properties. Needed is a rapid response method for measuring average moisture content of the moving product stream at the drier outlet, which could be used for automatic control of operating variables such as air speed and temperature to result in continuous production of a product with optimum moisture content. For some commodities, possibly alfalfa, monitoring the surface temperature of the product in the exit stream may be as effective as measuring moisture.

### **III. Proteins and Related Nitrogen Compounds**

The rapid measurement of essential amino acids and proteins is one of our primary needs in this area of analysis. We also need improved methods for determining nutritional availability of proteins and amino acids, for hydrolyzing proteins prior to their analysis, and for differentiating between vegetable and animal proteins; and we need to eliminate environmental pollution caused by mercury waste from the Kjeldahl method for nitrogen determination.

#### **A. RAPID MEASUREMENT OF ESSENTIAL AMINO ACIDS AND PROTEINS**

Even the most fully automated systems presently available do not satisfy requirements for use in plant breeding, crop management, and marketing of cereal grains. There is need for rapid, fairly reliable, simple, in-

expensive micromethods. These methods might involve measurements of behavior of component electrons, protons, neutrons, or a combination of them without destruction of the sample or individual grains which could then be used by plant breeders. Other possibilities include component-specific dyes which may then be detected by colorimetry. With non-destructive dyes the grain may be used for improvement purposes. Still another possibility is development of newer chemical procedures that could be adapted to automation. Automated devices should be simple to operate and chemical procedures reliable and fairly accurate. A small amount of accuracy could be sacrificed for speed of analysis. More refined techniques, many of which are now available, could be used to confirm the discoveries from the more rapid methods.

Rapid methods for the determination of sulfur-containing essential amino acids would be extremely useful because of the nutritive significance of these amino acids. A chemical method for methionine and cystine would provide information on the relative nutritive value of foodstuffs without relying on long and tedious animal feeding studies.

Present methods are complicated by the instability of these two amino acids during acid hydrolysis, especially in the presence of carbohydrates. So extensive is the destruction of these amino acids that hydrolyzates of foods upon chromatography generally exhibit no measurable cystine peak and methionine is partially oxidized to the sulfoxide.

The most accurate method to quantitatively determine the amount of sulfur-containing amino acids in food products requires oxidation of the products with performic acid to convert cystine to cysteic acid and methionine to methionine sulfone. These reaction products are stable during acid hydrolysis of the food proteins to their amino acids, but this extra oxidation step adds an extra day or two to the analytical procedure.

A more rapid and accurate method to determine these amino acids or to stabilize them to hydrolysis, especially in the presence of carbohydrates would be extremely useful to the food industry in establishing nutritive value or specific deficiencies in foodstuffs.

Similarly, more rapid and reliable methods are needed for tryptophan. Tryptophan is an essential amino acid which is one of the limiting amino acids in many proteins. Because of its acid lability, tryptophan cannot be determined on the usual hydrolyzates used for determination of other amino acids by ion exchange and gas-liquid chromatographic (GLC) techniques. Alternative methods of hydrolysis of proteins such as alkali or enzymatic methods have been developed. Also, many colorimetric reagents have been used. However, most of these procedures are time consuming, and a review of the literature shows that the reported values vary

widely. For example, reported tryptophan values of defatted soybean meal range from 1.09 to 1.6 grams per 16 grams nitrogen. As a result, modifications of the tryptophan analysis procedures are continually appearing in the literature without any assurance that the values obtained by these procedures are accurate or that they can be duplicated in other laboratories.

Problems with high-color blanks, poor recovery of added tryptophan, and interfering substances in the reagents have been reported [2,3]. Procedures which involve enzyme pretreatment of the protein may require as much as 96 hours of hydrolysis to release the peptide-bound tryptophan from soy protein [3].

Accurate and rapid procedures for tryptophan are especially needed to evaluate the biological value of proteins by the system adopted by Food and Agricultural Organization of the United Nations [4] where each essential amino acid is reported as a proportion of the total of essential amino acids (milligram per gram total essential amino acids). These values are then corrected to percentages of hen's egg essential amino acid pattern. Development of a method for determination of an acid-stable derivative of tryptophan along with the other amino acids in an acid hydrolyzate of a protein would seem to be a worthwhile undertaking for the future.

Rapid, accurate analytical methods are needed for determining the protein content of dairy products. Payment for milk in commerce is made on the basis of its fat content. This assigns a premium to a component which is at an economic disadvantage with respect to vegetable fats. Milk fat is also frequently implicated in health problems when consumed in excessive amounts. If a rapid, accurate method for milk protein determinations was available, payment might also be made on the basis of protein content. This would tend to publicize a component in milk which is qualitatively superior to many other proteins, and de-emphasize high-fat milk. It might also lower the minimum fat contents specified for many dairy products.

Rapid response methods for control of protein content in mixed feeds would be important economically. Formulation of mixed feeds by the larger feed suppliers is now done on a least-cost basis by a computer program utilizing as input data current market prices and nutritional values of the various components of the ingredients and specified minimum nutritional values of the product. Because of variations in composition of various lots of ingredients purchased from different growers, it would be economically advantageous to the feed mixer to be able to allow for such variations during the mixing process rather than use proportions which ensure minimum specified protein and energy contents based on spot



analyses of ingredients. Such a capability would also result in more uniform nutritional quality of product with potential advantages to the purchaser.

Moisture and protein contents are the key values to be measured continuously in streams of ingredients being mixed. This information would be instantly introduced to a computer process control system which would automatically adjust the proportions of the ingredient streams being mixed. The methods currently used for indicating protein measure nitrogen content, which is satisfactory for this purpose. However, methods now in use are not fast enough to be used for essentially instantaneous control. Closest approach to date appears to be the automated determination of nitrogen by high-energy neutron activation in which there is a lag time of 5 minutes from introduction of sample to availability of result [5].

## B. NUTRITIONAL AVAILABILITY OF PROTEINS AND AMINO ACIDS

Chemical analysis for nutrients in foods and feeds often yields results which exceed the actual nutrient value of the sample. This discrepancy arises from the fact that the digestive system of the animal does not completely digest some nutrients due to the chemical bonds present. Nutrient unavailability can be created during plant growth as exemplified by bound niacin in corn, or it can result from processing as with lysine losses due to heating. Present methods of determining the extent of digestibility and availability involve feeding studies. These studies are generally carried out using rats. Other animals are sometimes employed. Feeding studies are time consuming and can be expensive depending on the animal used. Kohler *et al.* [6] have reported *in vitro* method for determining protein digestibility in wheat millfeeds using a proteolytic enzyme digestion followed by treatment with chick pancreas amylase. This method or a variation of it must be evaluated as to its general applicability. A rapid, reproducible laboratory procedure that is correlated to each animal type would be an outstanding contribution to the science of nutrition.

Lysine and methionine are the essential amino acids most often found to be "limiting" in grain and other vegetable food and feed proteins; *i.e.*, these compounds are most likely to be present in smaller quantities than other essential amino acids relative to the amounts required for good nutrition. In particular, lysine has been added to bulgur and other wheat-



based foods supplied in nutritional aid programs to developing countries. The total lysine content of proteins is apparently reliably measured by the standard amino acid analytical methods, but not all of the lysine present is nutritionally available to the consumers of the protein-containing foods or feeds. Much evidence indicates that nutritional availability of lysine is correlated with the amount of lysine present with free  $\epsilon$ -amino groups.

A method has been developed for measuring the lysine-free  $\epsilon$ -amino content of proteins through formation of the 1-fluoro-2,4-dinitrobenzene derivative. This method has been widely used as a measure of nutritionally available lysine, but it appears to be only partially satisfactory and is less so for vegetable than animal proteins. Another method employs 2,4,6-trinitrobenzene sulfonic acid, a reagent which also reacts with free primary amino acid groups to form a colored derivative. Although more rapid than the fluorodinitrobenzene procedure, this method is subject to interference from hexosamines leading to erroneous results.

There is also much evidence that not all the methionine content of proteins is nutritionally available, but so far there is no chemical method to eliminate the need for bioassay measurement of available methionine. Accordingly there is need for reliable chemical methods which can be used in food and feed formulation and quality control work for determining nutritionally available lysine and methionine contents of proteins. Such methods must show good correlation with results obtained by the laborious animal feeding studies.

### C. OTHER PROBLEMS

A critical problem in determining total amino acids in proteins of foods and feeds is the lack of a hydrolysis procedure that yields amino acids truly proportionate to their composition in the unhydrolyzed protein. Hydrolysis is usually conducted with constant boiling hydrochloric acid at elevated temperature and, even under the most carefully controlled conditions, some destruction of labile amino acids occurs. At the same time some peptide linkages are peculiarly resistant to hydrolysis, so that prolonged periods of heating are required to break them completely. Thus, tryptophan is usually destroyed entirely, cystine is altered significantly, serine and threonine are degraded at more or less predictable rates, and other amino acids—notably methionine and tyrosine—may be somewhat labile. On the other hand, long periods of heating are needed to liberate valine and isoleucine to the fullest extent.

In the analysis of purified proteins the problem has been solved reasonably well but at the cost of additional time-consuming procedures. Special methods for tryptophan, cystine, and methionine are available. Analysis of acid hydrolyzates prepared by heating for different periods of time permits extrapolation to zero time for good estimates of serine and threonine content, while the results obtained from long-term hydrolyzates represent maximal yields for valine and isoleucine.

The problem is more serious in the analysis of foods and feeds containing relatively little protein. The presence in foods and feeds of carbohydrates, especially those like arabinose or pectin which yield furfuraldehyde when heated with acid, enhances the destruction of several amino acids. Another consequence is diminished recovery of other amino acids because of formation of insoluble humin under these conditions. Attempts have been made to minimize these effects by hydrolyzing foods or feeds in the absence of oxygen and with large excesses of acid, and by filtering off and washing insoluble humin before analysis of the hydrolyzate. Another approach has been to prepare protein concentrates from the foodstuff for analysis.

Numerous investigators have attempted to bypass the problems of acid hydrolysis by the use of enzymatic hydrolyses. These efforts have not yet provided a quantitatively satisfactory method. There is, however, within this approach the potential which may make it possible to pour a solubilized protein solution onto the top of a column and collect the constituent amino acids from the bottom. The use of a layered column of immobilized enzymes could once more make science fiction a reality. In addition to the use of a number of different proteolytic enzymes, it would also be necessary to shift the optimum pH of activity through the chemical reaction used in immobilization to a pH value mutually compatible with the different enzymes and with the solubilized protein. This is not beyond the realm of possibility.

Another need in protein analysis is a means of differentiating between vegetable and animal proteins. This problem is gaining in importance because of the substitution of vegetable for animal protein in products that formerly contained only animal protein. Rapid, accurate, economical procedures are needed not only by regulatory agencies to test and verify product compliance, but also by manufacturers for quality control to avoid economic losses. A recent method, not quite in vogue, utilizes immune absorption techniques to prepare species specific antisera for use in immunochemical detection of species adulterants formerly impossible due to cross reactivity [7]. A simple, more rapid, and accurate immunochemical test would be of value.

We also need a method for distinguishing between endogenous and plant origin of fecal nitrogen. The digestibility of feed protein is usually estimated by determining the difference in the ration nitrogen and the fecal output nitrogen. Since the nitrogen of feces arises from endogenous excretions and some tissue material, as well as the residual nitrogen in the undigested ration, the true digestibility of the ration or feed cannot be determined directly. Furthermore, the nitrogen content of bacterial cells which contain nitrogen originating in the ration and extracted from it, but residing in the excreted bacterial cell, represents another fraction of interest. The elucidation of the true digestibility and efficiency of absorbing nitrogen from various feed components would be greatly enhanced if the several nitrogen components could be distinguished in the fecal material.

The final need in the area of protein analysis is for elimination of environmental pollution caused by discharge of mercury in the waste of Kjeldahl nitrogen determinations. The official method for determining nitrogen in foods, feed, fertilizers, tobacco, and coloring materials is the Kjeldahl test, which requires the use of mercuric oxide as catalyst. Analyses for nitrogen are made in thousands of laboratories. Based on estimates that 80 percent of the approximately 224,000 pounds of mercury consumed annually in the United States for catalytic purposes [8] is used for such tests, this source produces 180,000 pounds of waste mercury per year. Either an alternate, noncontaminating catalyst or method must be developed, or mercury must be recovered from present analytical wastes.

#### IV. Lipids

The chemical determination of lipids has lagged far behind that of other chemical analyses. Unless scientists are specifically conducting studies in this area, they tend to ignore the lipid content of biological materials because of the cost and amount of equipment involved, time required, use of solvents that require special handling procedures, and the limited number of analyses that can be conducted per day. It is felt that this analytical area has suffered because of the lack of technology. A rapid and simple method of analysis is needed and, if developed, should go far in encouraging additional research in the lipid area.

##### A. RAPID MEASUREMENT OF LIPIDS AND FATTY ACIDS

A specific need that we would like to mention is for a rapid determination of fat in corn grits. The market for brewers' grits is an important out-



let for the corn dry-milling industry. Brewers specify the maximum allowable content of extractable fat, usually about 1 percent in the grits they purchase. During milling operations, corn is passed through a Beall degerminator mill to fracture the corn and separate the high-oil germ fraction from the low-oil grit fraction. The brewers' grits fraction may be loaded directly into a rail car for shipment. However, before the grits can be shipped they must be analyzed for fat content. The current extraction procedure for this analysis requires several hours. The millers need a rapid procedure that can be performed in a few minutes. Prompt analytical information would allow changes to be made in milling operations when fat content of product exceeds specification and would allow the miller to produce more of the higher priced grit product. Wide-line nuclear magnetic resonance (NMR) has been proposed as a rapid method for determining oil content, but this technique has not been shown to have the required accuracy at low-fat levels.

Our plant breeders need a nondestructive method for fatty acid determination in connection with their efforts to breed oilseeds with specified fatty acid composition. Problems of flavor stability, such as rancidity, are associated with oxidation of unsaturated fatty acids in vegetable oils.

Striking progress has been made in changing the fatty acid composition of rapeseed oil and safflower oil by plant breeding. In these cases, the desired changes in fatty acids were simply inherited. Success was achieved despite the necessity of destroying the seed in order to obtain a sample for analysis by GLC.

Soybean oil contains about 7 percent linolenic acid. Sharp reduction or elimination of this acid would probably significantly increase stability of soybean oil. Unfortunately, soybean germ plasm free of linolenic acid has not been discovered. It is therefore necessary for the plant breeder to use various techniques to apply "selection pressure" in favor of those genetic types which have lower than normal linolenic acid. Thus he might approach his goal of an acceptable level of linolenic acid in stepwise fashion.

To do so he needs a nondestructive method of fatty acid analysis. Such a method would avoid destruction of the very seeds with the most desirable attributes. In GLC analysis the desirable seeds are identified only after they have been destroyed.

The plant breeder ordinarily must examine thousands of plants in order to find the rare one with desired qualities. The method must therefore be simple, accurate, and suitable for rapid analysis of many samples.

NMR is a nearly perfect tool for oil analysis for the breeder of oilseed crops. A method or instrument of similar capabilities and moderate cost is needed for fatty acids.

We anticipate a possible need in the not too distant future for rapid, simple, economical methods to measure the degree of saturation of fatty acids and the amount of cholesterol in meat, dairy, and egg products; baked goods; and fats and oils. This need is arising from the national concern about dietary cholesterol and saturated fats. One of the recommendations in the "Report of Inter-Society Commission for Heart Disease Resources" [9] was that efforts be made to "modernize regulations on labeling and definition of foods so that consumers can identify nutrient contents such as the amount and type of fat and cholesterol." If more detailed labeling practices should be initiated, there will be a need for massive routine analysis of food for type and amount of lipid. Present techniques, which involve solvent extraction of lipids, ascertaining the nature of fatty acids by methylation and GLC and determination of cholesterol spectrophotometrically, will not meet the future need for rapid, simple, economical methods.

An incompletely answered question in this field is, "What are the physiological effects of ingesting *trans* isomers of polyunsaturated lipids in humans and animals?" Some evidence of detrimental effects from *trans* polyunsaturated lipids has been obtained from laboratory animal feeding experiments. Laboratory rats fed diets high in *trans* polyunsaturated lipids showed increased blood clotting time and lowered prothrombin levels in their blood. Because of the possibility of other damaging effects from *trans* polyunsaturated lipids, it is important to have a rapid, sensitive analytical technique for estimating the level of each individual isomeric polyunsaturated lipid present in processed foods and edible vegetable oils. Also, such a technique could be used to study *trans* polyunsaturated lipid levels as related to processing conditions in edible vegetable oils and lipid-containing foods.

The current analytical technique used for determining *cis/trans* ratios in edible vegetable oils depends on two independent measurements. The iodine number gives an estimate of the total carbon-carbon double bond concentration in a vegetable oil. In order to ascertain the *trans* isomer content of an edible vegetable oil, it is first necessary to make a series of infrared analyses of standard mixtures of methyl elaidate in carbon disulfide. A standard curve is then constructed by plotting the depth of the  $970\text{-cm}^{-1}$  band against concentration of the elaidate solutions. Then an infrared analysis of the edible oil is made and the depth of the  $970\text{-cm}^{-1}$  band is obtained from the recorded spectra. The concentration of the *trans* carbon-carbon double bonds, expressed as concentration of methyl elaidate in the oil, is obtained from the standard curve.



The current analytical procedure does not give detailed information on the levels of each isomeric *trans* polyunsaturated lipid present. In order to intelligently plan biological experiments and understand the food processing conditions responsible for *trans* polyunsaturated lipid production, a new analytical technique is needed.

Another problem associated with vegetable oil processing is the need to know how much natural linolenate and linoleate remain in soybean oil after partial hydrogenation. Partially hydrogenated soybean oil contains as much as several percent of linolenate and linoleate isomers different from the naturally occurring forms. In such oils the content of the natural isomers can now only be determined by the alkali isomerization method. Because of the nutritional significance of natural linoleate and linolenate and because most soybean oil is hydrogenated, the alkali isomerization method of analysis is used extensively. GLC does not distinguish between various nonconjugated isomers.

The Official Alkali Isomerization Method is laborious and requires about 2 man-hours per sample. A recently developed new procedure combines isomerization and GLC for determining linoleic acid, other conjugatable dienes and nonconjugatable dienes in triglyceride esters, simple esters, and fatty acid mixtures [10]. Compared to the conventional alkali isomerization-spectrometric method there are advantages of ease, microscale capability, speed, and versatility. However, this new procedure is not suitable for samples containing large amounts of triene and is not proposed as a replacement for the more accurate alkali isomerization-spectrometric method. It is suggested as a technique for the analyst having a GLC and needing to determine linoleic acid in oil which contains a small amount of triene. For process control uses and especially for research studies, it would be very desirable to have available a rapid, accurate method for determining the content of natural linolenate and linoleate in partially hydrogenated soybean oil.

## B. COMPOSITIONAL CHANGES IN LIPIDS

All oils and lipid-containing food products can suffer flavor and odor deterioration due to oxidation of the lipids. Better methods are needed for monitoring production and storage of such food products. This need will intensify if food distributors are required to include on the labels of their products the date of manufacture and/or the date when the product should be removed from the store shelf. Urgently needed is a reliable, rapid predictive test which will indicate the approximate shelf life of a product.

Tests run at temperatures considerably higher than normal are generally not accurate. Simple Warburg techniques are useful, but until now, the Warburg apparatus has not been automated in a commercially successful instrument. There has been developed recently a new mini-gasimeter which can continuously record gas uptake or evolution [11]. It is not yet known if this type of measurement (using only mild temperature increase, exposure to light, and exposure to 100 percent oxygen in place of air) will give a reasonably accurate automated predictive test for shelf life of lipid-containing products.

In addition a method is needed to determine how far a food material has progressed toward becoming unpalatable or objectionable because of oxidative rancidity. Use of such a test could allow the manufacturer or distributor to extend the period during which a given lot of product could be marketed. This problem is a very difficult one because the human nose and palate are more sensitive than most common instrumental techniques.

In the preservation of meat and meat products, oxidative deterioration of the lipids is by far the most important source of quality loss. Autoxidation of the unsaturated fatty acids and decomposition of the resulting hydroperoxides produce aldehydes which are principally responsible for the rancid flavors that develop. The flavor effects of individual aldehydes is cumulative so that traces of several individuals have a serious total effect. Thus, quantitative isolation and identification of these aldehydes have been considered important to measurement of rancidity, and an understanding of the chemistry involved.

Development and application of chromatographic procedures have enabled the identification and measurement of the aldehydes as 2,4-dinitrophenylhydrazones. The aldehydes which are characteristic of a given oxidized unsaturated fatty acid have been identified and measured. Peroxide decomposition studies have shown that with respect to aldehyde formation the scission of hydroperoxides is a selective process which is influenced by the proximity of a double bond. This fact explains the predominance of certain aldehydes which is not supported by the amounts of the precursor hydroperoxide isomers known to be present.

We still know very little of the nature and rates of reaction of the intermediate present. However, the complexity of hydroperoxide decomposition reactions is emphasized by the fact that scission decomposition to aldehydes represents only a small proportion of the total hydroperoxide decomposition. The nature of the other decomposition products is mostly not known. A smaller volatile dicarbonyl fraction has been detected in oxidized pork fat from adipose tissue. These volatile dicarbonyl compounds have not been identified. However, they are probably vicinal and of

moderate chain length. A nonvolatile dicarbonyl fraction is believed to exist, but this has not been explored in meat fats. Study of the dicarbonyl compounds generated in oxidized fat is a useful line of investigation.

Meat contains two fat or lipid systems. Nonpolar lipids, which are represented by the triglycerides, compose most of the adipose tissue and the fat marbling in the lean tissue. Autoxidation of the triglycerides is fairly well understood, and the methods referred to above are applicable. The second fat system is the polar lipids, represented principally by the phospholipids. Little is known about the oxidation of phospholipids, although in some meat products they are a source of rapid deterioration.

In uncooked meat cuts the polar lipids are stable. They are apparently protected by meat enzymes in the lean tissue, of which the phospholipids are an integral part. The enzyme systems maintain a reducing state which prevents oxidation. Processing which inactivates the enzymes renders the polar lipids very vulnerable to oxidative attack. These lipids contain much more polyunsaturated fatty acids than the triglycerides. In the meat tissue environment, hydroperoxides do not accumulate and apparently are immediately decomposed by the catalysts present. Identification and measurement of the carbonyls and other rancid flavor compounds formed and their probable interaction products with amino acids and proteins are unexplored analytical problems in meat research.

When fats are heated in air, as in the case of deep fat frying with vegetable oils, they are partially transformed into volatile chain-scission products, to nonvolatile oxidized derivatives, to cyclic substances, and to dimers and polymers. If the heating or oxidation is severe, the fats lose part of their nutritive value, probably because they contain substantial levels of unabsorbable polymers. Further thermal or oxidative abuse may cause the fat to become toxic. Certain cyclic derivatives and the oxidized dimers are probably among the toxic compounds.

Since the cyclic compounds formed at low levels during the heating of a fat are among the few components for which reasonable, convincing evidence of actual toxicity has been obtained in studies with laboratory animals, it would seem most desirable to have a dependable method for their determination.

Many tests already exist for measuring either fat quality or the level of deterioration products in fat. However, a dependable method for the determination of cyclic esters is not available. The separation of the whole complex of cyclic and branched compounds from noncyclized fatty acids has always depended upon urea adduction which is not suitable for use in an analytical method. The development of an analytical method for quan-

tatively determining cyclic esters in heated oil would aid in resolving their contribution to the toxicity of heated fats.

We have a second problem related to cyclic substances, but this problem concerns naturally occurring cyclopropenoid fatty acids in contrast to cyclic acids that are formed in heated oils. Some 10 years ago a program was initiated to develop analytical methods for the quantitative determination of the cyclopropenoid fatty acid content of cottonseed oil. This was an urgent problem at that time because of the potential biological implications of these cyclopropenoid components on animal and human well-being. As a result of this research program a number of analytical methods were developed for determining the amount of cyclopropenoid material present in oils and meals, on both a micro- and semimicroscale. These included infrared spectrophotometric methods [12,13], a number of titration methods utilizing a titrant of HBr in acetic acid [14-16] and modified Halphen methods [17].

Analytical procedures now are needed for determining the composition of the reaction products formed during processing and after ingestion of cyclopropenoid material.

Another problem is related to the quantitative analysis of heat-sensitive fatty derivatives that are surface active. These derivatives usually have molecular weights between about 350 and 2,000. They usually are in mixtures which contain many isomers. They are easily degraded by heat, acids, and alkalis. Examples of these fatty derivatives are esters of sucrose, glucosides, polyglycerols, and other polyols. Sucrose and a given fatty acid can form up to 255 different compounds (8 monoesters, 28 diesters, 56 triesters, 70 tetraesters, 56 pentaesters, 28 hexaesters, 8 heptaesters, and 1 octaester).

TLC of a sucrose ester product produces a series of spots. Qualitatively, the groups of mono- and diesters can be identified with certainty, but the others cannot. Quantification of the different types of esters is even more difficult. Ester spots on a silicic acid plate can be made visible by any one of a number of reagents, one of the better being a solution of urea and phosphoric acid. However, the determination of the amount of ester in a given spot is far from accurate. For that matter, all quantitative determinations of spots on TLC plates are relatively inaccurate. Measurements of the amounts of pigments, either by transmission or reflection densitometry, do not give the necessary accuracy even assuming that the pigments formed in proportion to the amounts of compound present.



### C. OTHER PROBLEMS

There is a great need for a procedure to distinguish various fats and oils from one another and when in admixture. This information is necessary for use in normal trading and to detect adulteration of fats and oils, which is a problem that has been of considerable concern to the U.S. Food and Drug Administration.

Standards have been in use for many years as a basis for trade within countries and between countries. These standards are based on classical determinations of iodine values, refractive index, saponification value, relative density, and others. Employing all these analyses, many oils could not be distinguished one from another, let alone in admixture.

The Codex Committee on Fats and Oils, sponsored by the Joint FAO/WHO Codex Alimentarius Commission, has recently proposed identification of fats and oils from their fatty acid composition as determined by GLC. This is a considerable step forward which will reduce the number of fats and oils that cannot be distinguished and affords a better means of detecting admixtures.

At the present time, improved procedures in GLC and TLC allow considerably more information to be determined about the biological source of the samples of fats and oils. Determination of the amount and chain length of the triglycerides present and the amount and type of sterols present in the sample apparently will greatly reduce the number of oils that cannot be distinguished.

From limited information, it appears that each vegetable oil has specific sterols in a ratio to each other that exhibit little change, regardless of the environment during growth and the maturity of the plant. Various animal fats appear to have somewhat different triglyceride combinations but similar sterols. Much more work needs to be undertaken along these lines, but should these preliminary findings prove to be true, there would be very few fats or oils that could not be identified; and indeed, it would be possible to identify many in admixture.

What other types of analyses can be used to supplement the present GLC-TLC approach to this problem?

The presence of dispersed plastics in rendered fats, particularly rendered inedible tallow, presents an increasingly urgent problem. Plastic materials, predominantly polyethylene, become evenly distributed throughout the fat during the rendering process. The polymeric material is usually present in about 50 ppm concentration as microcrystalline particles in solid fat or as a fine haze in melted fat. Although the problem has



been intensified by the use of polyethylene drum liners that may find their way into the finished animal fat, it is generally thought that the major source of polymeric materials may well be packaging films that enter into the rendering tank when off-condition packaged meat is sold to the renderers. It is economically infeasible to remove packaging film from large quantities of meat prepackaged in retail cuts.

The haze which dispersed plastics impart on melted tallow is objectionable to producers of fat-enriched feeds, possibly mainly for aesthetic reasons. More serious are the problems caused to the fatty acid producer by the presence of these polymers. More than 600 million pounds of inedible animal fats are used for the production of fatty acids each year in this country. During the fat splitting process the polymeric materials accumulate in the pumps and splitting towers, causing frequent and serious equipment failure.

The economic removal of dispersed plastics from animal fats represents an urgent problem. Even more urgent, however, is the development of a rapid and dependable analytical method capable of detecting the presence of plastic materials in concentrations ranging from  $< 5$  to 100 ppm. Both the Fatty Acid Producers Council and the National Renderers' Association are actively concerned with this problem and are supporting research aimed at solving it. Several analytical methods have been proposed, but none has as yet been found which is rapid, accurate, and applicable to low concentration ranges. One method which has been proposed is based on extraction of the fat with petroleum ether, filtration of insoluble material, and solution of the latter in hot benzene, reprecipitation of the polymeric material with methanol, and filtration and weighing of the polymer. Confirmation of the polymer as polyethylene is carried out by infrared analysis. Other methods depending on infrared spectroscopy and on GLC have also been proposed.

What is needed is a simple, direct, sensitive, and rapid method which gives reproducible results. Such a method should be capable of being carried out by personnel having limited technical training.

Another problem to be solved is that of sequential analysis of lipids in membranes of various cells in nature. Cell membranes consist of lipoprotein which can be isolated in fairly pure state. The protein moiety can be analyzed by various techniques available to protein chemists, but the lipid moiety cannot be analyzed. The coordination of phospholipids, cholesterol, small amounts of free fatty acid and triglyceride to form the lipid portion of lipoprotein is poorly understood. All that is really known is the fact that the lipids are not covalently bound to protein and that they are extractable from lipoproteins. The various forms of the lipids can be

separated into the major classes and quantitatively analyzed. But the manner in which the lipids are organized in the cell membrane is unknown. No methodology is presently known for solving this problem. An understanding of membrane structure is critical for comprehension of transport mechanisms across cell membranes. Many of the aging and disease processes of cells are intimately related to this problem. The analytical chemist has yet to devise a method to solve this problem.

### V. Fibrous Constituents of Plants

Information on crude fiber content of feeds and cereals is used extensively in the milling and cereal industry. Crude fiber values are reported along with such other values as total protein, crude fat, and moisture contents on most packaged dry cereals. This analysis is thus related to all programs involving cereal products. The present methods involve a high degree of empiricism, and the total crude fiber may vary considerably unless official procedures are rigorously followed. Even day-to-day checks by the same operator on the same sample sometimes yield a high relative standard deviation. According to the standard procedures of American Oil Chemists' Society (AOCS), Association of Official Analytical Chemists (AOAC), and American Association of Cereal Chemists (AACC), one man can accomplish no more than three to four analyses per day. All of these methods are basically quite similar. The sample is subjected to treatment in boiling sulfuric acid (1.25 grams/100 cm<sup>3</sup> water), followed by a slow and tedious filtration through a cloth of exacting specifications. The remaining material is subsequently treated with boiling sodium hydroxide (1.25 grams/100 cm<sup>3</sup> water), and filtered again, followed by extensive hot water washing. The remaining material is dried and weighed. This material must be ashed so that the mineral content may be subtracted, since it is not considered part of the crude fiber.

Since crude fiber content is an indicator of the quality of feeds and processed foods, a crude fiber analysis has been considered a necessity by the milling and cereal industry. The requirements of a more desirable method would be greater reproducibility, less empiricism, and certainly, from the economic point of view, fewer man-hours per sample.

Determination of cellulose and/or lignin contents of plant materials and their derived products is important because this information is essential in agronomic and genetic studies, process control, maintenance of product

quality, and appraisal of economic potential of fibrous materials, especially for the pulp and paper industry. The widely divergent backgrounds and sources of lignocellulosics contributes to great dissimilarity in compositional characteristics and in presence and extent of extraneous substances that interfere with the determination of either cellulose or lignin. Accurate measure of the contents of both in original plants, materials in process, and in pulps and papers is essential to the Department's program to develop processes for producing commercially acceptable pulps and papers from kenaf and other nonwood plant materials.

Present analytical techniques are empirical and generally depend upon selective solution of the undesired constituents and contaminants so that either cellulose or lignin remains and can be measured gravimetrically. Because of the types of materials examined and their histories, elaborate time-consuming schemes are necessary to eliminate undesired components. Frequently, purification procedures that must be employed contribute to degradation products, as exemplified by beta and gamma cellulose. A number of so-called direct techniques are also employed. Oxidation-reduction equivalents, viscosity of solutions from metallo-ammonium or amine complexes, and optical densities determined under closely controlled conditions are some of the procedures employed. All suffer from lack of suitable reference standards to correlate with lignin or cellulose contents in materials examined.

Rapidity, accuracy, and economy in analyses are essential if we expect to decrease the gap between raw material supplies and demand for lignocellulosic fibers in the years immediately ahead. Scanning electron microscopy, differential scanning calorimetry, electron spin resonance, wide-band NMR, and other developments of the past decade offer exciting new possibilities for achieving these objectives in measuring cellulose and lignin in plant materials at all stages of processing.

## VI. Nutrients and Their Availability

Concern with nutritional status of various segments of our population has called attention to the need for accurate data on quantity, form, and availability of trace minerals and other nutrients, such as vitamins in soybean and cereal products. Literature values for these important nutrients often vary widely which indicates that the analytical results are suspect. In some instances analytical values are nonexistent [18].

In addition to total amounts of a given nutrient, it is necessary to know the relative amounts of various forms of the nutrient that may be present, because biological availability varies with different forms. For example, minerals such as iron may occur free, bound to protein, and as ferric phytate. With some nutrients the forms in which they exist are still unknown; thus, there is no methodology available for their determination. To determine the distribution of other nutrients such as phosphorus in inorganic, acid-soluble, phosphatide and phytate forms [19], tedious extraction procedures followed by ashing and colorimetric methods are used. Because soybean and cereal products are used in a large number of food products, there is a need to develop more rapid and more accurate methods that could be applied to the various food items now available.

There is also an imperative need for reliable data on the amounts in foods of three nutrients—folacin, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>, whose dietary importance has only recently been recognized. This information is essential for monitoring the nutritional adequacy of our present-day foods, many of which are being used in highly processed forms. Since 1968, when the Food and Nutrition Board of the NAS-NRC first issued recommendations for the amounts of these essential B-vitamins, the demand for data on the content of these vitamins in foods has greatly intensified. As vitamin B<sub>6</sub> occurs in foods in two and often three forms, which future research may find to differ in biological value for man, determinations for each of the three forms are needed.

A major problem in the determination of the vitamin content of foods is the complete release of the bound forms. Analysts tend to adopt a single procedure for all types of foods regardless of the diversity in physical and chemical composition. Collaborative studies are needed to determine the best hydrolysis procedures for each of these vitamins and for each type of food.

Currently the best data available for these three B-vitamins in foods, including the different forms of vitamin B<sub>6</sub>, are from microbiological assays. These procedures are much too time consuming and expensive to be practical. Alternative methods are needed for determining the content of these B-vitamins in foods. Although some effort has been exerted in this direction, the challenge remains.

Several attempts have been made to devise chemical methods for these vitamins. In the 1950's, Japanese workers reported on a procedure involving ion-exchange separation and a fluorometric assay of the vitamin B<sub>6</sub> components in foods. To our knowledge, however, this work has not been duplicated. When the eluates used by our Human Nutrition Laboratory for the microbiological assay were tried fluorometrically, greatly in-



terfering fluorescent substances were found present. Further work would be needed to obtain fractions suitable for fluorometric measurement of the three components of vitamin B<sub>6</sub> in foods.

For folacin in foods, a fluorometric method has been described recently in a Russian publication [20]. The authors reported that their folacin values for the foods tested—cheese, carrots, and cabbage—compared well with their results from microbiological assays with *Streptococcus fecalis*. This fluorometric procedure may have potential for use. It would need to be tried with a wider variety of foods and should be compared with microbiological assays using *Lactobacillus casei*, the organism presently preferred.

If satisfactory alternative methods cannot be developed, far more standardization is essential in the microbiological assays as currently used. The vitamin B<sub>6</sub> assay developed in our Human Nutrition Laboratory, and now designated "Official, First Action" by the AOAC, represents a significant advancement. Total vitamin B<sub>6</sub> is obtained by an ion-exchange separation of the three forms and microbiological assay of the three eluates. Thus the net effect of the destruction and conversion of one form to another can be followed during the processing and storage of foods. Few processed foods have been assayed for the three components of vitamin B<sub>6</sub>. Ways of speeding up this cumbersome procedure would be highly desirable.

A recent report describes a semiautomated and a fully automated method for estimating folacin activity in human serum [21]. The antibiotic chloramphenicol base and a chloramphenicol-resistant strain of the preferred test organism eliminate the need for sterilizing the test material. Attempts might be made to adapt this method to the analysis of foods.

Microbiological methods for vitamin B<sub>12</sub> continue to present problems. Agreement is lacking on many of the details in methodology including the choice of the test organism. If no other means of determining vitamin B<sub>12</sub> can be devised, the microbiological assay should be thoroughly reinvestigated.

Part of the problem with microbial determinations is caused by instability of test organisms. The reliability of the method can be improved by use of the same batch of frozen inoculum over a period of time. The organism for the vitamin B<sub>12</sub> assay was found to remain stable for over 1 year when held frozen in liquid nitrogen. Another procedure for the liquid-nitrogen preservation of the organism for the vitamin B<sub>6</sub> assay, when used with additional special techniques, resulted in reduction of the



incubation time from the usual 16-22 hours to 6 hours. Confirmation of this technique is needed.

If organisms can be stabilized, some of the organisms discarded in the past should be retested. The search should continue for organisms of equal or greater sensitivity which require less time of the analyst.

The need also exists for a standardized method of determining the absolute assimilability of iron by humans and animals. Recent food consumption surveys have revealed widespread deficiencies of iron in the diets of many Americans [22]. Such surveys normally assume a 10 percent absorption of iron from food. Biological assimilability data are frequently obtained by testing small animals. Investigators making such tests have reported figures for the bioabsorption of dietary iron ranging from 5 to 90 percent. Different iron compounds and food combinations are known to affect the bioavailability of iron. Also such a wide variation may reflect the lack of a standardized method. In view of the current efforts being made to enrich a variety of foods with iron, a reliable, standardized method of measuring the absolute assimilability of iron by both humans and animals is needed.

## VII. Seed and Plant Constituents

The Department of Agriculture engages in extensive efforts, research and otherwise, to promote industrial, nonfood uses of farm products; and the problem of suitable analytical methods for constituents that are important in such uses is very relevant to these efforts. A desired constituent may be extracted or otherwise removed from the commodity in question for use or, alternatively, may impart essential properties for industrial applications of the agricultural product *per se*. In either event, accurate knowledge of the amount, availability, and functionality of the constituent in the product is important to ensure necessary yield or performance for the processor and/or user and a fair price for the producer. This information can also aid in defining practices to be recommended for maintaining consistent and high contents of the valuable ingredients, levels of which may vary significantly with genetic history of the crop, growth conditions (geography, soil type, climate, agronomic treatments), maturity and moisture content at harvest, and treatment after harvest.

Many examples could be cited, but three will serve to illustrate the general nature of the problem: (1) the content of the key acid in certain species of high-erucic acid oilseeds ranges from zero to approximately 60

percent depending primarily on the seed variety; (2) many oilseeds contain smaller quantities of highly unsaturated acids when grown in the South than when grown in cooler northern areas; (3) drying of corn at temperatures above 60 °C can create problems in the subsequent manufacture of starch from it. In these examples, present methods vary in adequacy and complexity. Extraction of the seed oil, conversion of the acids in it to methyl esters, and analysis of esters by GLC are the steps involved in determination of erucic acid content. A titration is used to determine iodine value, a measure of unsaturation. No better method than carrying out of a wet-milling operation in miniature has been developed for measuring the available starch in corn that may have been heated excessively.

Recent work with wide-line NMR is one example of the kind of modern techniques wherein the answer may lie. Single-step instrumental approaches could provide the needed speed and procedural simplicity to facilitate commerce and research progress involving the constituents and commodities in question. These approaches developed for the examples cited should be applicable with minor adaptation to related useful constituents; *e.g.*, hydroxy acids and epoxy acids in the case of seed oils, and amylose in the case of starch.

In the area of marketing, rapid methods of compositional analysis are needed for grain and oilseed grading and quality determination to facilitate commercial trading. These rapid methods should also be helpful in determining nutritional value of grain products such as feeds and in distinguishing varieties of grain, particularly wheat. Present methods involve visual inspection and laboratory testing for grading and certification. Carlots are held while this procedure is carried out. This practice is too slow for today's market activities. On-the-spot testing of grain prior to shipping would be beneficial to both farmer and tradesman. Delays while samples are sent to the laboratory for testing can no longer be tolerated. Therefore, the trend in analytical testing is toward nondestructive instrumental methods, preferably automated, to give a number of analyses in a few minutes. As soon as these methods can produce satisfactory accuracy they will, no doubt, be adapted to inspection procedures.

A related problem exists in the small lot grading of oilseeds, where there is need for rapid methods of compositional analysis. Since rapid methods are not available, all oilseeds in small lots are graded on an "as-is" basis, that is, on the average composition for the area concerned. Present chemical methods of grading oilseeds are applicable only to the trading of large lots, where the time and expense of analysis can be tolerated. Small lot grading must be rapid, simple, and inexpensive. Such

grading must be based on instrumentation, preferably of nondestructive nature. Our new infrared reflectance analysis of ground soybeans for determination of oil, moisture, and protein is a step in the right direction. This method must be further refined before it will meet all requirements. For other oilseeds (cottonseed, safflower, sunflower, and flax) other approaches may be required but in all probability should be based on an instrumental method of analysis.

We have another marketing problem that is arising because of the possible future packaging and storage of processed grain and other commodities in polyethylene-film containers instead of the paper and cotton bags that are now in use. The problem arises from the effect of the new moisture-impermeable film containers on the storability indicators that are now used.

Percent moisture content and fat acidity value are two of the most used chemical indicators of grain storability. After 1 month of unfavorable storage conditions, the percent moisture content for commodities stored in paper and cotton bags will normally rise severalfold and the fat acidity value will drop to near zero, concurrent with a loss of oil. For polyethylene-film containers stored under the same conditions, preliminary results indicate that percent moisture content does not increase significantly and the fat acidity value does not drop to near zero. Thus, there is a need for a rapid analytical indicator of grain storability for commodities stored in the new moisture-impermeable containers.

We also anticipate a need for rapid, simple tests to identify high-oil, high-lysine, and high-protein corn kernels when these corns become available on a commercial scale. These specialty corns will be sold, at least initially, on an identity preserved basis. Therefore, mixing with regular dent corn must be avoided during drying, storage, transport, and milling. Methodology apparently does not exist which would provide the elevator operator, shipper, or miller with tests for making on-the-spot identification of the new corn types.

In the area of plant physiology, we need nondestructive, highly portable, and self-contained methods and apparatus for analysis of plant constituents in studies on absorption, translocation, and metabolism of nutrients in plants. The nutrient status of a plant can be estimated by analysis of various plant parts. However, the plant-to-plant variability may be very great, partially or completely obscuring day-to-day variation. At present, tissue samples selected by empirically determined criteria are taken on some sort of schedule.

A nondestructive method would permit successive analyses of the same plant or tissue, thus removing errors due to plant-to-plant variability. Ap-

paratus should be portable and sturdy enough to move to field or greenhouse and should have modest power requirements.

Elements of interest are N, Ca, P, K, Mg, S, and the minor elements—Fe, Mn, B, Cu, Zn, and Mo. The minor elements may be present in amounts from a few parts per million to less than 1 ppm.

Methods are needed for identification and analysis of “active” fractions in plant cell contents. For instance, many plants store substantial quantities of protein in seeds or other tissues. Is such protein metabolically active? The plant scientist needs a rapid and unsophisticated means to address this question.

### VIII. Animal Products

Our analytical needs in this area can be conveniently grouped according to food and nonfood products. For food products, we are concerned with measurements of quality and of composition. Our needs in the area of nonfood animal products center around leather manufacturing.

#### A. MEAT QUALITY

The standards for quality grades of carcasses are based in part on the physiological age of the slaughter animal as determined subjectively by a trained meat grader. Degree of ossification of bones, darkness of myoglobin pigmentation, and texture of *Longissimus dorsi* are some of the factors used in ascertaining physiological age of the carcass. The meat industry would feel more confident of decisions placing carcasses in the top portion of the next lower grade if objective methods for quality grading were used. We developed a procedure in which the resonant frequency and/or the sonic velocity of the right metacarpal was related to the chronological age of Black Angus steers. This technique needs to be adapted for use with bones of the wholesale carcass. A rapid objective method is needed to determine the physiological changes which occur during maturation and thus provide an index of physiological age.

In order to certify the wholesomeness of meat from slaughter animals, we must be confident that the meat did not come from dead, dying, diseased, disabled, or drugged animals. We have found that determinations



of pH, myoglobin and hemoglobin-myoglobin ratio do not serve as criteria for unwholesomeness. Objective tests for detecting such meat are lacking.

Instrumentation is needed to measure metabolic changes in the live animal that are indicative of unwholesomeness in the carcass. Criteria which can relate changes in the composition of meat to the health of the slaughter animal from which it originated are also desirable.

Over the past several years, meat research workers and livestock breeders have made great strides in producing pork carcasses which contain a higher percentage of muscle and a lower percentage of fat. Accompanying this selection for a meat-type hog, there has developed in pigs a stress susceptibility which in some cases results in meat quality loss due to a pale, soft, and watery condition. In some cases, animals shudder and die before slaughter.

The occurrence of pale, soft, exudative (PSE) pork is usually reported from subjective observations of the loin muscle and exposed ham muscle or pH measurements on these muscles.

Improved antemortem methods, such as analysis of blood or biopsy samples, are needed for determining whether or not an animal is PSE prone. If these animals can be classified before slaughter, then they could be segregated into areas of greatest utility.

The Department of Agriculture requires that canned hams and similar nonsterile canned meats be heated during processing to temperatures high enough to inactivate certain viruses. In the case of imported meats especially, there is need for an analytical method which will allow Department inspectors to determine to what temperature a particular lot of canned meats was heated during processing.

A method based on observing the temperatures at which soluble proteins extracted from the ham coagulated was found to be an imprecise means, and a better method was needed. The heat inactivation of acid phosphatases has been found by workers both in Europe and in the U.S. Department of Agriculture to give an estimate of the thermal history of the canned meat which for 95 percent of the samples should be within  $\pm 2.4$  °F. of the actual temperature. The actual analytical procedure consists of extracting the acid phosphatases, allowing the extract to react with the substrate disodium phenyl phosphate, and measuring the phenol released by its reaction with 2,6-dibromoquinonechlorimide. Although the acid phosphatase method is a considerable improvement on earlier methods, an accurate means of determining the thermal history of canned products over wide temperature ranges is still one of our unsolved analytical problems.



## B. COMPOSITION OF MEAT AND DAIRY PRODUCTS

Consumer interest in the composition of meat is becoming increasingly more of a factor in the kind and amount purchased and consumed in the home. Years of study have shown that a number of factors related to the final quality of the product can be controlled by selective breeding and nutrition. These results were obtained from research studies of the dressed carcass which are not only time consuming but too late to be helpful to the meat animal breeder and feeder. It has been shown the antipyrine method for estimating composition, especially fatness in live beef animals, is satisfactory for determining fatness in that species, but not in swine or sheep. It is a long and involved procedure that requires trained technicians. A rapid, but accurate, analytical chemical method to determine the composition of live animals is urgently needed.

Compositional studies of slaughter animals have been carried out for many years, but have proven to be of limited value because (1) samples do not always relate well with total animal population, (2) sampling procedure is destructive, and (3) large samples are required. Accepted procedures are macro or semimicro in nature and only partly automated. A need exists for semimicro- and micromethods that are automated and applicable to specific systems such as the musculature, the circulatory, and secretive systems.

Federal regulations presently limit the amount of fat that can be added to meat products such as frankfurters. Buying and selling meat scraps and ground meat on the basis of accurate estimates of fat content can result in more equitable distribution of the market dollar, and some assurance that the consumer is not paying lean meat prices for fat. Connective tissue is less digestible than fibrillarsarcoplasmic protein and can be present in fresh ground meat in amounts approaching 20 percent. Knowledge about amounts and distribution of connective tissue within a carcass and fresh meat derived from it would aid in the determination of (1) meat quality and (2) which muscles should be converted into further processed products to increase collagen digestibility by heat treatment.

Currently, fat content can be determined by ether extract, or methanol chloroform methods, but these procedures are rather time consuming. A commercially available Digital Fat Controller, which computes specific gravity of the meat sample, can be used to estimate fat content within 0.1 percent in just a few minutes. This instrument is relatively expensive (about \$10,000) and cannot be used on meat samples below 32 °F. Another instrument employs x-rays for estimating fat content. A third in-

strument in the planning stage will use infrared for measurement of fat and moisture in meat. Connective tissue can be determined using a histometric point-counter method or can be characterized by chemical methodology.

A spectrophotometric technique for determining moisture, oil, and protein in soybeans is being developed in Department laboratories. Once developed, its applicability to meat will be determined since such a method would be most welcome. Spectrophotometric and sonic methods for determining connective tissue will be evaluated as an approach to non-destructive, rapid instrumentation.

Some processes used in the manufacture of dairy products such as evaporation, precipitation, coagulation, and crystallization involve rapid changes in the concentrations of total solids, proteins, and lactose. Quick methods for determination of these components are needed so that quality control can be more accurately and efficiently maintained.

Reliable data on cholesterol in foods are urgently needed. Control of dietary cholesterol has emerged as an essential part of treatment in certain cardiovascular problems. In fact, some medical groups have been pressing for curtailment or, at least, moderation in cholesterol content of normal as well as therapeutic diets, particularly for such vulnerable groups as the adult male.

Cholesterol is present only in foods of animal origin. The main sources in the American diet are eggs, poultry, dairy products, fish and seafood, and meat and meat products. For most of these sources only a few samples have been analyzed. Frequently the range reported by different investigators for different samples of the same kind of food is so wide that an average value would have doubtful significance and if used would be misleading. Information is fragmentary or lacking and is therefore urgently needed on the cholesterol content of such important items as cooked meats, luncheon meats, most varieties of cheeses, and for low-fat dairy products such as skim milk and uncreamed cottage cheese. Such values as are reported for some samples of skim milk and some cheeses are more than 100 percent higher than the values reported by other investigators. Content of cholesterol reported by various investigators for salmon and oysters show even greater spreads, being in the order of 300 percent for salmon and 1,000 percent for oysters.

Reliable, reproducible methods are necessary before such problems as normal sample variation and species and seasonal differences can be studied and used in deriving guidance materials on cholesterol content of foods and diet. The methodology must be tailored to be quantitative measurement of total cholesterol—free and ester—in a wide range of foods

and food mixtures. Methods satisfactory for use in clinical analyses of blood and serum may require extensive modification to be suitable for analyzing foods, with their far wider range of problems in extraction and interferences.

Various combinations of solvents and different extracting times are in use for the extraction of total lipids from a food prior to determining cholesterol. This lack of uniformity may account for some of the wide range in values observed. Special attention is needed on procedures for completely extracting cholesterol from products with little total lipid and low cholesterol content such as skim milk, lean meats, and the many mixed foods containing one or more animal products.

The principal method of cholesterol determination used to date on lipid extracts from foods relies on the Liebermann-Burchard color reaction in which cholesterol gives a color when combined with sulfuric acid, chloroform, and acetic anhydride. Since this reaction is not totally specific for cholesterol, a preliminary digitonin or tomatine precipitation is usually made on the crude unsaponifiable extract to eliminate noncholesterol sterols. The tomatine procedure is newer and more specific for cholesterol than the digitonin, but both are subject to many interferences. Other procedures of assay include the AOAC bromination, fluorometric, and gravimetric digitonin methods.

A recently developed technique [23] for determining cholesterol by GLC, while more specific than traditional colorimetric methods, has had almost no application to analysis of foods. As presently published, the GLC method requires about 2.5 grams of extracted fat for the analysis. The weight of sample of low-fat foods required to provide this amount of fat could be critical in some research. Therefore, specific standardized methods for determining cholesterol in much smaller amounts of lipid extract are needed.

The widespread use of diethylstilbesterol in livestock production makes knowledge of its metabolism imperative. Although there is vast literature on the use of diethylstilbesterol, a total metabolic scheme is not known in any animal species. The amount and nature of biological residues in edible meats and the biological effects of these residues on the animals have not been well characterized. Conventional chromatographic separation and colorimetric procedures are available for macroquantities of diethylstilbesterol. GC procedures are being applied to diethylstilbesterol determination but adequate methodology does not as yet exist.

A matter of concern related to the use of antibiotics in animal production is the possibility that residues of these compounds may be found in the edible tissues of livestock after they have been processed for human

consumption. This possibility exists, whether antibiotics have been routinely used for growth stimulation or for therapeutic purposes. Antibiotic residues in animal tissues have been determined by means of microbiological assays. These microbiological methods are relatively slow and laborious, as well as being specific only for the compound in question. There is a need for simple and rapid analytical procedures which would identify not only the parent compound but also possible metabolic breakdown products which might accumulate in various body tissues. Such procedures would permit a more accurate determination of the fate of antibiotics in the animal after ingestion or injection and also allow for the monitoring of larger numbers of meat samples in a consumer protection program.

### C. HIDES AND LEATHER

Analytical methods are needed to account quantitatively for all of the sulfur-containing compounds in the lime-sulfide unhairing of hides. The tanning industry is having considerable difficulty in reducing the quantity of pollutants that it produces. One of the severe problems is the biological oxygen demand (BOD) and sulfide content of the effluent from the unhairing system. The reactions involved are quite complex and have never been fully studied because of the lack of analytical methods suitable to follow the five inorganic (sulfide, polysulfide, hydrosulfide, sulfite, and thiosulfate) and four organic (cystine, cystiene, lanthionine, and methyl mercaptan) sulfur compounds suspected to be involved.

Sulfide is used up by the excess cystine in the hair, but may be regenerated as sulfide or polysulfide by a second reaction which yields lanthionine. Whether these reactions are directly involved in the hair-loosening or hair-dissolving step has never been established. Efficiency in the use of sulfide might be increased if more were known of the actual hair-loosening reaction. The present analytical methods are not sufficient to clearly establish all of the competing and serial reactions involved.

If the rate of the hair-loosening reaction could be increased at the expense of the hair-dissolving reaction, a considerable decrease in the BOD load and a reduction in the sulfide requirement might result. Since the same problem affects tanners throughout the world this is a problem of international significance.

The introduction of pickled cattlehides as a marketable commodity has been hindered by a lack of adequate means of quality control and enforceable specification parameters.



At present there is no standard procedure for determining the acid (pH 2) and salt (10 to 15 percent) contents of pickled hides. The acid is usually sulfuric and the salt, sodium chloride, although formic, acetic, and paratoluene sulfonic acids and sodium sulfate have also been proposed.

Ashing is unsatisfactory since some of the components will be volatilized or partially altered. Quantitative extraction from the hide involves lengthly manipulation. The determination of pH values on a 10-fold water extract after 12 or 24 hours has little reliability as a measure of acidity.

Present attempts to assay hides utilize electrodialysis, surface specific ion electrodes, and conductivity and capacitative measurements to determine total electrolyte contents and microelectrode probing to determine stratigraphic distribution of electrolytes within hides.

Department research has led to the commercial use of glutaraldehyde in the tanning of leather. Our research also disclosed that the aldehyde is bound irreversibly and not liberated upon hydrolysis of the tanned protein. However, hydrolysis of glutaraldehyde-tanned hide substance does produce ultraviolet absorbing species (maximum at 265 nm) which correlate with bound glutaraldehyde as measured by loss of aldehyde from the tanning solution. This method of analysis is adequate for research purposes where the tanned hide substance may be analyzed before the finishing operations necessary to produce the finished leathers. Many materials used in finishing also absorb in this region so that this method for detecting and estimating bound glutaraldehyde is not applicable to finished leathers. This is a disadvantage from the standpoint of control of a new commercial tanning process and of specification of this new leather.

Fundamental research to establish the nature of the ultraviolet absorbing species can be expected to contribute to the solution of this analytical problem. Because the tanning industry is lacking in research facilities and technical staff, sophisticated and expensive techniques are to be avoided.

## IX. Sensory Characteristics of Foods

Nearly all food research investigations require taste-panel evaluation of food products or intermediates as a primary criterion of the efficacy of raw material or processing variations. Flavor and odor evaluations by taste panel require a large enough group of tasters so that reliable statistical evaluation of results can be made because of the variability of sensory evaluations. For these and other reasons, food research involves con-



siderable expense. Instrumental methods should be developed to supplement and partially supplant taste-panel methods as an economic measure. In certain of the beverage industries, for example, GLC analysis has been used to advantage in this connection. However, it appears that flavor compounds frequently are present in amounts far below the detection limits of present-day instrumental sensors, since the most potent flavor and off-flavor compounds are often detected by odor or taste, not instrumentally. Hence, further development of instrumental methods seems warranted.

Present knowledge of the essential components of cooked aromas, as well as the methods of estimation, are inadequate to provide processing plant controls. Subjective odor panels are time consuming and expensive and not adaptable to a continuous plant monitoring of the total production. Solution of this general problem will first require well coordinated chemical, analytical, and sensory evaluation of the commercially important cooked food aromas. When the essential components and their critical proportions are established, then relatively simple plant control methods can be sought which will permit monitoring optimum flavor development in the further processing of meat, poultry, and other heat-processed foods.

Another important sensory characteristic of foods is "eye appeal," which is still measured largely by subjective techniques. Apparently, the wealth of spectrophotometric equipment in the modern laboratory can tell the scientist little about the spectrum of light reaching the eye from the surface of a peach, chocolate fudge, or a beef roast. Certainly the consumer easily notes color differences and reacts decisively to changes in the visible region that the photometers cannot detect or describe.

In a typical instance, research on fortifying milk with iron to overcome fairly widespread incipient anemias in certain population groups has been hindered by the fact that the added iron causes color changes in some foods in which milk is traditionally used, such as cocoa. The eye easily detects off-colored cocoa, but the finest reflective spectrophotometers are blind to these changes. Obviously, much more effort could be spent in the study of light reflected from complex surfaces of multiphasic systems which have a measurable degree of translucence.

## **X. Microorganisms in Foods**

There is a constant and ever-growing need to assess and control microbial growth in our food products, in order to conserve our foods and

provide a wholesome, safe food supply with an adequate shelf life. Presently available and used methods of estimating total viable bacteria almost always involve culturing and counting, which are laborious and time-consuming operations. As a result, products are either unduly delayed, or they move through channels of processing and distribution without a current record of their bacterial load.

Attempts so far to provide alternative chemical methods, such as dye reduction methods, have fallen short of their goals. A workable method should be general enough to react to and measure all viable microorganisms, and yet be specific enough to exclude all dead cells and foreign organic matter. This is an extremely difficult goal for the analytical biochemist, and probably would involve a basic search for suitable transient intermediary metabolites to serve as the reactive chemical indicator of total viable bacteria present. Such compounds might be sought in the general area of nucleotides and other biochemical organic phosphates.

At the present time, standard cultural and serological techniques for the isolation and identification of *Salmonella* require at least 4 days for positive identification. Since the methods are complex and time consuming, there is general agreement that more rapid and reliable means of detection are needed. Rapid methods are particularly needed for the assessment of sanitation in food processing plants.

Department scientists have developed a new culture medium and testing procedure for *Salmonella* in dairy products which combines the advantages of a newly developed cultural procedure with the specificity of absorbed antisera conjugated with fluorescein isothiocyanate. The completed test for *Salmonella* requires about 24 hours.

Positive presumptive evidence of *Salmonella* is obtained in 18 to 24 hours by growth in a selective enrichment medium. *Salmonellae* are indicated by the medium changing color from red to black and/or yellow. The medium contains a broad spectrum antibiotic for bacterial selectivity and the enzyme trypsin, which digests milk casein and clarifies the medium. Confirmation of *Salmonella* in the 18 to 24 hours' growth medium is determined by the fluorescent antibody technique. Dairy products assayed by the new procedure have included raw and pasteurized milks, milk concentrates, nonfat dry milk, whey, and cheeses. Although the new procedure reduces the time for assay by one-fourth, a still more rapid procedure is highly desirable for monitoring food processing operations.

Occasionally, canned whole tomatoes and some aseptically canned products such as pea soup suffer flat sour spoilage in which acid is produced, off-flavor develops, and eventually some gas may be formed

due to bacterial action involving chiefly *Bacillus stearothermophilus* or *Bacillus coagulans*. Fortunately, the incidence of such spoilage is infrequent, the spoiled product is not toxic, and the likelihood of the spoiled product being eaten is small. Nonetheless, the problem results in both economic loss and consumer dissatisfaction. At present, there is no simple, objective, reliable method of detecting flat sour spoilage without opening the can. One consequence of such spoilage is a marked decrease in the viscosity of viscous products. This change may provide one possible basis for developing a nondestructive test for flat sour spoilage in canned products.

## XI. Animal Health

Analytical problems in this area stem from the Department's surveillance efforts to detect foreign animal diseases and our regulatory and control program on veterinary biologicals.

### A. FOREIGN ANIMAL DISEASES

There are numerous animal diseases exotic to the United States that would be devastating to our livestock and poultry industry should they become established. The concept endorsed by U.S. officials is that any outbreak of a foreign animal disease that would result in significant loss to the U.S. livestock industry must be quickly detected and eradicated promptly. To accomplish this, a surveillance program has been established, and each reported case suspicious of being a foreign animal disease is investigated by a veterinarian with special training in the diagnosis of foreign animal diseases. Laboratory specimens such as tissues, oesophageal-pharyngeal fluids, blood, and serum are collected and sent to appropriate laboratories for serological diagnosis. The time required for a tentative diagnosis can vary from hours up to several days and sometimes weeks. Since many of these diseases spread in an explosive manner, this lengthy testing procedure may permit a disease to spread widely before eradication procedures can be initiated. More rapid tests, such as analytical chemical methods, that could be developed to quickly and economically determine the presence of a foreign animal disease would allow control measures to be effected before the disease has a chance to spread widely.

Imported animal products have a disease-producing potential for domestic livestock of the United States. Therefore, an accurate as possible determination must be made that these products do not contain animal disease-causing microorganisms. Many animal products, including meat, are visually inspected at ports of entry to determine whether the products have been processed in conformance with Department regulations and directives. At times, it is not possible to make a clear-cut decision on the basis of visual examination. In many of these instances, samples are submitted to the laboratory for analysis for final determination. Development of rapid, simple analytical methods which could be used at the point of entry by laymen to determine the safety of a material would be beneficial. In addition to providing an immediate determination, test results would provide backup support to the decisions of the inspectors.

Similarly, the chemical identification of foreign animal disease organisms would be beneficial in the Department's program of regulating the importation and interstate movement of organisms capable of causing disease in animals. Problems often arise when considering the importation of an organism because of the presence of an animal disease in the country of origin which is not known to exist in the United States. Although the organism which would be imported may be rather innocuous, fear of contamination of the culture, or the culture media, with a foreign animal disease agent can result in the importation being refused or being allowed only under stringent restrictions. These restrictions hamper some scientific exploration in the United States.

## B. VETERINARY BIOLOGICALS

One of the objectives of the Department is to conduct a regulatory and control program for assuring the consumer that all veterinary biologicals are safe, pure, and potent. There are several important analytical chemistry problems that need to be solved in order to better meet this objective. Three analytical problems that are of great interest to us are: (1) the development of improved methods for the purification, isolation, and identification of the active components of veterinary biologicals; (2) the development of analytical methods that can be used for the evaluation of fluorescent antibody reagents which are used as an aid in the identification and diagnosis of animal diseases; and (3) the development of analytical methods which permit correlation of chemical structure and biological function and thus promote the development and use of synthetic antigens.



### *1. Active Components of Veterinary Biologicals*

Many of our chemical problems in this area are bioanalytical in nature and require the correlation of chemical results with biological tests. Presently, we perform many product evaluations using *in vitro* biological systems and others which can only be made by using host or laboratory animals. These tests adequately evaluate the products but are often expensive and time consuming to perform. Most of the biological products are very heterogeneous and contain a large percentage of material other than the active components. The bacterins and toxoids contain many nonessential metabolic products along with the active components, and the viral products contain large amounts of growth-supporting material such as the residue from egg or tissue culture menstrum. The presence of these residues have detrimental effects on any direct analytical approach to quantitating the efficacy of these products.

One of the problems we face is to develop suitable purification procedures that will permit more direct *in vitro* tests which can be used to verify the safety, purity, and potency of these products. Several purification procedures such as dialysis, ultrafiltration, and gel filtration are now being used to help solve this problem.

### *2. Fluorescent Antibody Reagents*

We are developing a program for the evaluation and control of fluorescent antibody (FA) conjugates which are used for the identification and diagnosis of animal diseases. FA, as the name implies, are specific antibodies which are conjugated to a fluorochrome. The fluorescent labeled antibody permits the microscopist to readily identify the specific antigen present in a bacterial smear or tissue culture preparation.

We presently produce and evaluate a number of specific FA reagents both for our own use and for the use of biological producers. These reagents are used solely as reagents for the evaluation of veterinary biologicals. A much larger number of FA reagents are commercially produced for use in the diagnosis of animal diseases.

The procedures now being used for the evaluation of FA conjugates are very time consuming and require the microscopic examination of many slides. Evaluation is further complicated and the work increased manyfold by the requirement that conjugates must be specific and give a negative reaction for a great number of similar antigens.

A major analytical problem is the development of a rapid and sensitive method for the evaluation of FA conjugates used for the identification of animal diseases. One method that is under consideration involves the use of a flow-through laser photofluorometer. In this method, the antigenic cells, either viral laden tissue cells or bacterial cells, are mixed with the FA conjugate, incubated, and then passed through a microflow-cell in a continuous stream. The cells would be counted, sized, and their relative fluorescence determined. An index could then be developed to correlate relative fluorescence with the number of antigenic cells. The details of this method have not been worked out, but the general concept seems quite feasible.

### *3. Synthetic Antigens*

Most veterinary biologicals are very heterogeneous and contain many substances other than the active components. Many biologicals contain nonessential metabolic byproducts as well as large amounts of growth-promoting substances which have not been used by the organism. The active components have an extremely high level of biological activity and yet only represent a very small amount of the total composition of the product. Many toxins and allergins demonstrate biological activity in the nanogram and picogram range of concentration. These two classes of biological products probably will be among the first to be sufficiently well characterized that they could be replaced by synthetic antigens.

A great deal of effort has been made to purify and characterize several bacterial toxins. However, most of the work has been concentrated on tetanus, diphtheria, and botulinum toxins with relatively little work on toxins from other organisms. One of the main problems involved with purification and characterization procedures is the lability of toxin during processing. Most toxins are quite labile and readily degrade when they are separated from their native environment. Presently most commercial toxoids, chemically denatured toxins, are marketed as a mixture of detoxified toxins, other metabolic products, and substances used to promote growth of the organisms. The presence of such a large amount of nonspecific substances puts an undue burden on the animal to be immunized as it causes the immunological system to produce antibodies against all of these substances.

Allergins, such as tuberculin, are another class of products that could be sufficiently well characterized that a synthetic homologous antigen could be produced. Tuberculin is presently used as a cell-free filtrate con-

taining all the metabolic products of the organism as well as the nutrients required for its growth. An improved product is presently being evaluated for use in animals, and it consists of only the "purified protein derivative" (PPD) of the culture filtrate with most of the other metabolites and unused media constituents removed during processing. Although it is called a "purified protein," PPD is actually a group of proteins. It has been shown that the tuberculin PPD for use in human medicine contains a mixture of polymers. The basic component of the polymer has been identified as a small molecule with an estimated molecular weight of 7,000 to 10,000.

The fact that many toxins and the basic component of tuberculin have molecular weights in the 5,000 to 15,000 range enhances the feasibility that someday they may be produced synthetically. Technology is now available that will permit synthesis of compounds of this size, as evidenced by the synthesis of insulin and ribonuclease. The main problem facing us now is to purify the products and to perform the basic analytical work which will identify their structural characteristics. Once the structural characteristics have been described, it will be necessary to correlate the structure with biological function, and then we should be able to modify the structure of these components in order to obtain the necessary immunizing effect. The advent of synthetic antigens is probably many years in the future, but now we need to make our start on the analytical work that is the necessary forerunner of such an event.

## **XII. Biologically Active Substances**

Our interests in this area are varied, because Department programs deal with some aspects of the whole spectrum of human, animal, and plant life. We would like to discuss some problems related to the following substances: (1) indigenous plant constituents, (2) mycotoxins, (3) plant growth regulators, (4) pesticides, and (5) nitrosamines.

### **A. INDIGENOUS PLANT CONSTITUENTS**

Compositional studies of plant products under our new crops program have reminded us of the wide distribution of phytochemical substances potentially harmful to animals or man. Familiar examples are solanine in

potatoes, tomatin in tomatoes, thioglucosides in crucifers, lathyrus factors in certain legumes, indospicine and  $\beta$ -nitropropionic acid in *Indigofera*, oxalic acid in spinach, and cyanoglucosides in almonds, pecans, and tapioca. There is little or no information to indicate that any of these compounds are so distributed and concentrated as to be harmful to animals or man when the plant materials containing them are used in ways that are conventional at present. However, there is always the danger that new varieties, agricultural practices, or methods of processing may concentrate the undesirable principles beyond safe levels.

Present analytical methods generally involve some form of extraction and usually one or more fractionation steps applied to the extract to increase the concentration of the substance in question. Then, it is detected and estimated in varying degrees of quantitateness by colorimetry or some form of chromatography or spectroscopy. This multiplicity of steps is time consuming, and each step contributes to the error range or magnitude of uncertainty in the final answer. Opportunities for marked improvement should exist in procedures analogous to the highly automated ones currently used for amino acids and sugars.

Specifically, a need exists for simple and reliable methods to quantitate the various steroidal alkaloids present in edible Solanaceous crops. There has been a recent trend to use wild and uncultivated relatives of tomato, potato, pepper, and eggplant in varietal development programs. These wild relatives of the edible Solanaceous crops offer excellent sources of pest resistance. However, the introduction of new genes from uncultivated species of *Solanum* presents a problem to plant breeders because some of these uncultivated species are not edible due to their alkaloid content. It is conceivable that the use of these species in breeding programs could result in the release of new commercial varieties that contain alkaloid components not present in our older commercial varieties or varieties that contain higher contents of the alkaloids which are normally found in our edible Solanaceous crops.

For example, *Solanum chacoense* has been used in some potato breeding programs as a source of pest resistance. This wild relative of potatoes contains a new class of steroidal alkaloids, the leptines, in addition to solanine and chaconine, the glycoalkaloids found in normal potatoes. The leptines repel the Colorado potato beetle and its larvae, but because of the taste and pharmacological properties of the leptines, they may be undesirable in relation to food quality. At present, there is no simple screening procedure to determine the quantity of leptines and related water-soluble, steroidal alkaloids. A simple qualitative test for this class of compounds, as well as an improved quantitative method to determine



total alkaloids present in other edible Solanaceous crops, would greatly benefit the plant breeder, the food technologist, and, ultimately, the public.

This is but one example of the potential alkaloid problem involved in the breeding of Solanaceous crops and the need for improved analytical techniques for alkaloids in these crops. Breeding of Solanaceous crops involving gene transfer from inedible wild relatives poses a problem not generally encountered in breeding other agronomic crops. This problem can be readily resolved once we have the methodology to analyze our edible Solanaceous crops for these physiologically active alkaloids.

Another alkaloid is of interest in connection with fescue toxicity. Cattle grazing on tall fescue grass, *Festuca arundinaceae*, sometimes develop a lameness termed "fescue foot." The condition is characterized by a dry gangrene of the hind feet and tail. Cattle severely affected may die or recover with permanent defects of the feet. Animals mildly affected may recover completely following temporary lameness. Much research has been done to identify the toxic constituent or constituents with particular reference to toxic alkaloids. One such alkaloid, perloine, has been found to occur in the Kenwell variety of tall fescue and to peak at approximately 3,000 micrograms per gram dry weight for a period of 2 weeks. Up to 6,000 micrograms per gram dry weight has been found in some varieties of tall fescue. Perloine is in itself apparently not toxic to animals, but it can reduce the digestibility of cellulose in the animal and thus influence its performance. Further work needs to be done with perloine and possibly other alkaloids to determine their significance as toxic agents in forages. Techniques need to be developed to readily identify such agents and to analyze plant materials not only for their presence but for the quantitative levels produced.

A topic of increasing importance is the trypsin inhibitor content of soybean products, because food uses of soybean proteins are expanding. Present methods of determining trypsin inhibitor give varying results; thus, the absolute content of trypsin inhibitor in a given sample is still in doubt. Moreover, there are problems of reliability when the assay methods are applied to processed soybean products. A recent report [24] indicates that a significant level of residual trypsin inhibitor activity occurs in a commercial infant formula containing soy isolate. This infant formula gave a lower growth response when fed to rats than two other soy isolate-based formulas containing very low residual trypsin inhibitor activity. This problem is a matter of concern to soybean processors. Presently, four analytical methods are being used [25-28]. All of them are based on the principle of protein digestion and, consequently, are beset by

similar weaknesses. Soybean trypsin inhibitor (STI) is reported in relative terms, such as tryptic units inhibited or STI destroyed when compared to an arbitrary standard. Thus, in order to report STI activity in terms of milligrams per gram sample, absolute concentration of trypsin and protein substrate must be determined.

Chemical constituents in plants are affected by all aspects of the environment. Of particular interest is the recent knowledge that infection of alfalfa (*Medicago sativa* L.) by foliar pathogens results in accumulation of the estrogen, coumestrol, in plant tissues. Problems in animal performance have been attributed to feeding hay that is high in estrogen content. In some potent dehydrated alfalfa samples, coumestrol accounted for 90 percent or more of observed estrogenic activity. Interestingly, disease-free plants have about 2 ppm coumestrol while the coumestrol content of diseased plants often exceeds 500 ppm. No coumestrol is found in foliar pathogens that attack alfalfa. Information is needed to identify biologically active constituents associated with quality factors in forages. Also, we need to know the mechanism of biosynthesis of biologically active compounds like coumestrol and coumestans and the mode of interaction between plant and pathogen or other environmental variables. In other words, in a plant-disease relationship are other harmful constituents synthesized? Why is coumestrol synthesized instead of some other substance?

## B. MYCOTOXINS

Mycotoxins are a group of toxic metabolites—often carcinogens—produced by the action of molds on agricultural commodities and foods. The seriousness of the mycotoxin problem was not fully realized until the discovery of the aflatoxins, potent carcinogens, in 1960. Since then, a vast amount of research has been conducted to determine the extent of the aflatoxin problem. Figure 2 indicates the structural formulae of the aflatoxins with which we are primarily concerned. Presently, other mycotoxins are being studied to discover their significance in agriculture.

As part of the evaluation of the aflatoxin problem, qualitative and quantitative methods were developed to determine the incidence and levels of aflatoxins. Although procedures vary, basically each method consists of sampling, defatting, extracting, purifying, and estimating by TLC.

Currently, two methods are officially recommended for the determination of aflatoxin in peanuts by the AOAC. One involves an extraction

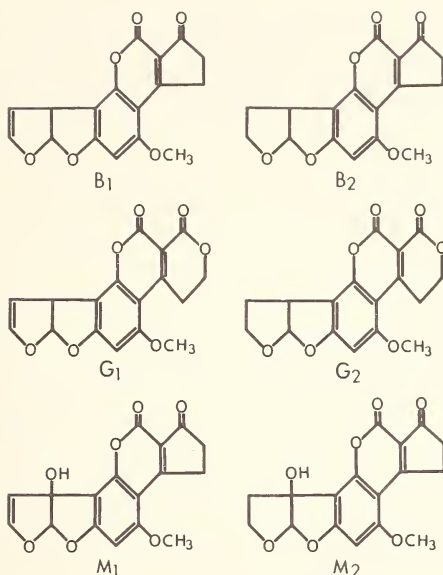


Figure 2. Structural formulae of aflatoxins.

with methanol-water and purification on Celite columns. The other method, known as the "CB procedure," recommended for peanuts, includes a chloroform extraction and a purification on silica gel columns. A modification of this method was used to screen export cargoes of corn for aflatoxin, ochratoxin, and zearalenone. In this study, ochratoxin was discovered for the first time as a natural contaminant.

As part of a study of mycotoxins in grains under the AOAC, studies are being conducted to establish a method of analysis for aflatoxins in corn and soybeans that can be officially recommended. Studies will be extended to include wheat, grain sorghum, oats, and barley in the future. These methods will probably be adopted by the AACC and the AOCS.

As other mycotoxins, such as sterigmatocystin, patulin, citrinin, penicillic acid, and tremorgens, take on new significance as carcinogens, suspected carcinogens, or toxins, the need for rapid, sensitive multitoxin methods increases. A multitoxin screening method for five toxins has been developed, but it lacks the sensitivity required for a quantitative method. More effort in this field is necessary.

Accurate and sensitive analysis for aflatoxins became available when automated fluorodensitometry was applied to evaluation of aflatoxin on TLC plates. This technique has been also applied to ochratoxin and

penicillic acid. GLC has been used to confirm the presence of zearalenone in corn and studied as a confirmatory test for patulin. This technique could be extended to other mycotoxins.

Aflatoxins  $M_1$  and  $M_2$  are carcinogens originally found in milk of cows fed aflatoxin-contaminated meal. The development of recommended procedures for determining  $M_1$  and  $M_2$  in agricultural commodities, particularly milk, has been delayed for two reasons. Aflatoxins  $M_1$  and  $M_2$  had not been separated on TLC plates, and supplies of toxins were not available for standards. Both of these problems have been solved by Department scientists, and it should now be possible to develop reliable analytical methods for these toxins.

Although methodology can be improved and hastened by automation, such as use of fluorodensitometers, mass spectrometers, and GLC, rapid detection in the field to avoid delays in moving commodities will require simplifications in methods at the sacrifice of accuracy. As new mycotoxins are discovered and evaluated, new methods will have to be developed.

In certain areas where cotton is grown under irrigation, invasion of bolls by *Aspergillus flavus* and the elaboration of high levels of aflatoxins in the seed is a serious problem. Even within a narrow geographical area the invasion is sporadic, with some fields heavily infested while others are relatively free of attack. The result is that ginned seed lots entering a cottonseed mill may vary widely in aflatoxin contamination.

Presently, the only detection system depends on the presence of yellowish fluorescent "cat eye" spots adhering to the short linted fibers when ginned seed are inspected under long wave ultraviolet illumination. Unfortunately, industry experience shows a poor correlation between cat eye fluorescence and aflatoxin contamination.

The availability of a rapid detection system whereby a representative sample of seed from an incoming truck or railway car could be analyzed within 5 to 15 minutes would allow contaminated seed to be diverted to special storage areas for separate, special processing and would offer further assurance of absence of contamination.

Improved methods for mycotoxins would also be extremely beneficial in the area of animal production. Most animal feeds are inhabited by a vast array of microorganisms, some of which are capable of producing materials which are highly toxic to animals and/or humans. Conditions are usually unfavorable either for the growth of the organisms or the production of the toxins. Therefore, the development of clinical symptoms from the ingestion of mycotoxins is not at all common. Because of the infrequent occurrence of clinical symptoms, knowledge concerning the occurrence of mycotoxin at subclinical levels is very incomplete. The possi-



bility that subclinical levels of mycotoxins are responsible for reduced levels of animal production is frequently posed. Improved methods of identifying and quantifying mycotoxins in feeds and animal products would be of immense help in locating and eliminating the potential losses from mycotoxins.

### C. PLANT GROWTH REGULATORS

Modern agricultural practice makes wide use of a variety of synthetic plant growth hormones, stimulants, and inhibitors to produce desirable modifications in the products we harvest. Our efforts are additionally influenced, however, by an indeterminate number of native or natural chemicals that powerfully influence plant growth.

Nowhere are these natural stimulants and inhibitors more important than in the problems associated with control of weeds. Persistent, hard-to-kill weeds depend, in many cases, upon efficient production of seeds, buds, and rootstocks or rhizomes that become dormant during adverse growing periods. The survival of these reproductive organs is dependent upon their efficient dormancy mechanism as well as the fruitfulness of the species. Subtle changes in biochemical intermediates, plant hormones, and the plant pigment phytochrome help to regulate dormancy. The problem of dormancy in plant tissues is an old one, and one that is critically important in making progress in control of weeds.

Control or modification of dormancy could hold the key to efficient removal of many noxious weeds which pollute the environment. However, this control cannot be achieved until and unless our special analytical problems can be solved. The gibberellins, kinins, auxins, ethylene, and other natural growth regulating compounds exert pronounced effects on plants or plant parts that are entering or emerging from the dormant state. They occur in small amounts, frequently as labile esters, complexes, or precursors. The many influential compounds involved vary greatly in chemical structure and reactivity, thus complicating analytical problems.

Detection in many cases depends on bioassay, making quantitation difficult. Modern chemical and physical methods of analysis may be employed where identity is known and standards are available for comparison. But in many cases related to dormancy, the identity of the inhibitor or promoter is uncertain and standards are not available.

The most critical phase of analysis is the initial extraction of the dormancy factors from the living tissue. Disruption of tissue during ex-

traction mixes the products being analyzed with a wide variety of natural products which may chemically modify the material the analyst is seeking. The extraction process may release or form substances that had no physiological significance in the plant until the maceration occurred.

The burden of analysis is placed upon nondestructive methods of extraction that will not result in coincidental modifications during extraction and separation.

One of the most difficult tasks we face in studies of tree physiology is the analysis of plant hormones. Plant hormones such as gibberellins, cytokinins, abscisic acid, indoleacetic acid, and ethylene influence virtually all phases of plant metabolism. They usually act in combination in determining important tree characteristics such as height of the plant, development of flowering, formation of seedless fruit, and resistance to stresses such as cold and drought.

Because the hormones act either in conjunction or in opposition, usually more than one has to be analyzed in studying any given process. For example, cytokinins, gibberellins, and indoles are important in fruit growth; gibberellins, cytokinins, and abscisic acid in bud break and seed dormancy; gibberellins and indoleacetic acid in stem growth.

There are presently available analytical techniques involving TLC, GLC, fluorometry, and spectropolarimetry to determine each of the hormones. However, the extraction procedure is elaborate for each hormone, and it is done separately for each compound. This makes the analysis of more than one hormone at any given time on a number of samples impossible. We need extraction and analytical techniques which allow us to extract more than one hormone at the same time. By developing such a method, we can expect to obtain much useful information about how to control plant metabolism.

Several chemicals are currently being evaluated for their effect on abscission as related to fruit harvest. Some materials hold the fruit on the tree; others induce abscission to aid in harvesting of the fruit. With constantly increasing labor costs, the ability to manipulate crops to enhance harvesting is essential.

To properly evaluate any chemical for use on an agricultural crop, the plant physiologist or biochemist needs to know the mode of action of the chemical in question. In most instances, he does not know how a chemical acts when he begins his initial evaluations but only the suspected response. Thus, as his evaluation proceeds, he usually conducts experiments designed to answer this question. Often this involves precise analysis of the specific chemical in tissue extracts. However, this technique is only superficial since it will not tell the scientist where the chemical is in

the tissue. Knowledge of the location of the chemical in the tissue is critical to understanding the mode of action. For instance, we know that cycloheximide induces abscission by influencing ethylene production which comes from tissue wounding. We do not know exactly which tissues are affected or the metabolic pathways involved.

One of the most challenging problems for the analytical chemist is to develop techniques for the analysis of these chemicals in intact tissue at the cell level. Techniques of this type would enable the physiologist to pinpoint the location and concentration of the chemical in the plant and relate this to the plant response obtained. Thus, the plants under test would not have to be sacrificed for analysis. In this regard, using an isotopically labeled chemical does not solve this problem in most instances.

## D. PESTICIDES

Problems in this area can be conveniently grouped into the following categories: (1) general problems, (2) pesticide complexes and metabolites, (3) problems associated with manner of application, and (4) structural basis of herbicide action.

### *1. General Problems*

We find that among our concerns of a general nature in the area of pesticides are analytical problems related to (1) confirmation of identity, (2) interfering chemicals, (3) new types of insecticides, (4) multicomponent analysis, and (5) pesticide disposal.

**a. Confirmation of Identity.**—Valuable as GLC has been in determining pesticide residues, the confirmation of identity, and the validation of quantitative measurements by independent means are still needed. The determination of GLC retention times on two or more columns or GLC followed by TLC can provide strong supportive evidence, but not necessarily unequivocal proof of identity.

Areas where research is needed included: (1) More specific GLC detectors for halogen and nitrogen.—The flame photometric detector is highly specific for phosphorus or sulfur and very sensitive (0.01 to 0.001 ppm). Electron affinity detectors currently used for halogen compounds are very sensitive but not selective. For nitrogen compounds, the best

methods now available are microcoulometric or electrolytic conductivity detection as ammonia (5 to 10 ng). (See also carbamates.) (2) Cheaper rapid scanning mass spectrometers and infrared spectrometers. — Present instruments are either too expensive or not sufficiently sensitive. (3) New methods of analysis. — One possibility is the plasma chromatograph in which an ionizing source charges molecules of a gaseous medium which react with trace organics to generate trace ions which are then separated according to their mobility (sensitivity reported at one part in  $10^{11}$ ).

**b. Interfering Chemicals.** — The very extensive use of polychlorinated biphenyls (PCB's) in industry has resulted in the appearance of these compounds in wildlife and in agricultural products. PCB's are incorporated into asphalts, rubber tires, paints, plastics, and a variety of other compounds. They have entered the air, water, and soil as a result of industrial and garbage smoke, automobile usage, and from industrial wastes. These compounds are similar in many respects to DDT, although analytical characterization is much more difficult. PCB's are an important source of interference in the chemical detection of chlorinated hydrocarbon pesticides. This is an example of an industrial pollutant, rather than a pesticide, which exerts adverse biological effects and produces residue contamination in agricultural products. This problem requires immediate attention.

Methodology is not presently adequate for separation of the PCB's from other chlorinated hydrocarbons and characterization. Pure samples of PCB's and polychlorinated triphenyls are needed. The very complex nature of the GLC patterns observed required better GLC systems to differentiate between compounds.

Another problem with interfering chemicals arises from trace contaminants in pesticides. Examination of technical pesticides often reveals many impurities. Each of these impurities has a different toxicity to plant and animal life. Identification and determination of trace contaminants in technical pesticide materials and formulations presents challenging problems to the analyst. For example, dibenzo-*p*-dioxins with less than five chlorine substituents have retention times on GLC columns similar to the chlorinated hydrocarbon insecticides. This may present difficulties in analysis.

Thus, there is need for the development of methods for the confirmation of trace amounts of hazardous substances in environmental and manufactured samples. Usage patterns, policy decisions, and even basic changes in the current direction of American agriculture may depend on the accuracy with which we can measure these impurities. Combined GLC/mass spectrometry appears to hold much promise here. To enhance



the value of this method for the solution of some specific problems, an improvement in interfacing or enrichment techniques to permit the sample to pass from GLC to mass spectrometer more efficiently is needed. The full potential of the technique can best be realized by computer storage and handling of acquired data to provide maximum information and rapid recognition of trace contaminants.

**c. New Types of Insecticides.**—Because of persistence the chlorinated hydrocarbon era is on its way out. Organophosphorus insecticides have been intensively investigated and will be widely used. Methods of detection are available. On the other hand, carbamates are being developed and used before good methods of analysis are available. Except for carbaryl, the carbamates must be reduced or derivatized for analysis, which means working with a variety of polar materials. A detector specific for nitrogen is urgently needed. Undoubtedly, other types of compounds will be developed for their degradability. Quantitative TLC with ultraviolet fluorescence may be a useful approach.

**d. Multicomponent Analysis.**—Rapid, comprehensive, and reliable procedures for samples of unknown history are of importance to assure safe food supplies. Some work has been done on GLC of phosphorus and sulfur-containing pesticides and metabolites in representative foods in which interfering substances are not excessive. Certain crops such as onions, turnips, and radishes are not amenable to this method.

**e. Pesticide Disposal.**—There is no completely safe, economical, and publicly accepted method of disposing of items such as used pesticide containers and used, dirty, or otherwise unwanted pesticide mixtures. Pesticide mixtures are currently supposed to be disposed of by dumping on ground at sites which are inaccessible to livestock and where such dumping will not contaminate surface or underground water supplies. These conditions of disposal are often difficult to meet because of lack of suitable disposal sites. Repeated disposal in the same area may result in pesticide buildup causing a spillover into the surrounding environment.

The qualitative and quantitative tests for pesticides are generally difficult to conduct as well as being costly and time consuming. For that reason, the potential for producing pesticide residues and the actual residue produced from dumping are usually estimated instead of being carefully calculated by analytical means. Better, more rapid, and more economical field tests for determining pesticide levels are needed. If such tests were available for use by the field operators, they could more accurately determine whether unsafe residues would be deposited as a result of disposition of pesticide or pesticide-bearing materials. The collection of a sample of well water for analysis is shown in Figure 3.



Figure 3. Well water sample obtained for analysis.

## *2. Pesticide Complexes and Metabolites*

Herbicides seldom exist unaltered in the soil, in plants, or in water to which they have been applied. We are aware that herbicides do degrade in the environment, resulting primarily in harmless metabolites. We are less well aware, however, of the marked tendency of many herbicides to exist in plants and soils as complexes, adducts, or conjugation products with natural materials. The existence of these herbicide combinations presents a significant challenge to the analyst. Figure 4 shows how a sample of soil is taken for measurement of pesticide residues.

A method of analysis that permits quantitative detection of the parent herbicide in extracts of crops or soil may not include all of the non-degraded herbicide present in the original sample. Herbicides may exist in biological systems as sugar esters, glycosides, amino acid adducts, and complexes with proteins and lignins. In fact, a single herbicide may occur in several of these forms simultaneously. For instance, the important herbicide (2,4-dichlorophenoxy)acetic acid (2,4-D) has been shown to complex with protein, to form an *O*-glucoside as well as a glucose ester, and to combine with amino acids. Chloramben (3-amino-2,5-dichlorobenzoic acid) is converted almost quantitatively to its *N*-glucoside in soybean



Figure 4. Soil sample being taken for measurement of pesticide residues.

roots and other plant tissues. Several carbanilate-base herbicides form bonds with lignins that are resistant to hydrolysis.

It is not sufficient simply to degrade the complexes and measure the released herbicide as is frequently done at present. The conditions of the herbicide in the plant and in the soil determine its persistence and residual phytotoxicity. Mammalian toxicity may also be significantly affected, and usually reduced, by the existence of the herbicide as a complex. Thus, for full understanding of the significance of the various states in which an herbicide can exist in nature, it is necessary to develop and use analytical techniques that will be quantitatively accurate, but that will reflect clearly the form in which the molecule exists at the time the sample is taken.

Another major problem of current interest and concern in the field of pesticide residue analysis is the development of suitable methods for isolation and quantitative determination of highly polar, water soluble, pesticide metabolites. Until recently, the major emphasis in residue analy-



sis has been placed on the isolation and quantitative determination of the nonpolar parent pesticide. The current extraction techniques and analytical procedures are often designed to examine only nonpolar parent pesticide residues to the exclusion of polar metabolites.

The justified and deep concern over what appears to be a substantial void in our information on the ultimate fate of pesticide molecules in our environment makes this limited approach to pesticide residue analysis no longer adequate or acceptable. The concentration, distribution, ultimate fate, and significance of polar pesticide metabolism in plants, animals, soil, and water have not been established. Indirect evidence now available indicates that the increased environmental burden of polar, halogenated, aromatic, and heterocyclic ring structures from pesticide applications may be significant over a reasonably long period of time.

The major polar metabolites of many pesticides have been isolated and identified, but rapid, reliable, and quantitative methods for the analysis of most of these metabolites have not been developed. The development of improved and selective extraction techniques for polar pesticide metabolites needs to be investigated. New and improved methods for the cleanup of extracts prior to quantitative analysis also need to be developed. The rapidly developing technique of high pressure liquid chromatography seems to offer many possibilities for the rapid cleanup of polar pesticide metabolites.

New and improved GLC techniques also need to be developed for the quantitative analysis of polar metabolites. Investigations in this area should include the development of improved column supports as well as new and improved methods for the quantitative conversion of polar metabolites to less polar or nonpolar derivatives before GLC analysis.

### *3. Problems Associated with Manner of Application*

Problems in this area are to be expected in view of the variety of ways in which pesticides can be used. We anticipate or have encountered problems related to (1) admixture of surfactants with herbicides, (2) testing of liquid fumigants, (3) encapsulation of insecticides in plastic, (4) application of chemicals in foams, and (5) application in sprays. Most of these problems are complex, and they probably will require a much more sophisticated approach to analysis than now exists. However, these problems cannot be ignored in view of the environmental implications of pesticide usage.



**a. Admixture of Surfactants with Herbicides.**—Additives of various types are becoming increasingly important in modern herbicide formulations. The surfactants added can completely change the activity of certain herbicides from one type of selectivity to another. In addition to the mixtures and blends of a variety of surfactants included in commercial formulations, the farmer frequently adds additional surfactant to enhance or modify activity or to improve herbicide solubility characteristics.

As a result, a large tonnage of surfactants is applied with herbicides to plants and soils each year. Potential persistence of these compounds varies, but the nonionics are most stable. Buildup of such materials in the soil can influence the availability, movement, and persistence of herbicides in the soil profile. Continued usage of herbicide-surfactant mixtures must be considered not only from the standpoint of alterations in herbicide persistence, but also from the standpoint of possible modifications in phytotoxicity patterns of herbicides applied later due to enhanced solubility in the soil.

Attention must be given to the possibility that accumulation of the surfactants themselves could result in harmful effects to plants, man, and animals. Many of the common surfactants have been shown to have adverse effects upon biological systems.

The analytical problems associated with surfactants and their use in weed control are very difficult. A large number of nonionic, anionic, cationic, and amphoteric products are available for use in formulations or for subsequent addition by the farmer. The identity of the surfactant in the formulation is seldom available, and analytical procedures presently employed are complicated, expensive, and not sufficiently specific for clear-cut identification.

Increasing use of surfactants with herbicides calls for a greater attention by analysts to problems associated with detection and quantitation of this important class of agricultural products.

**b. Testing of Liquid Fumigants.**—A liquid fumigant is applied to a stored product and sufficient time is allowed for the liquid to vaporize and distribute itself throughout the product. Samples of the vapor-air admixture are collected and transported to the laboratory for analyses by means of GLC.

If a researcher had a simple, rapid test by which to determine the concentrations of vapors in air, it would enable him to make immediate adjustments that could not be made otherwise. The equipment involved should not be large, bulky, or expensive.

**c. Encapsulation of Insecticides in Plastic.**—Because of problems as-

sociated with the use of persistent chlorinated hydrocarbons, ways are being sought to prolong the effective life of organic phosphate insecticides. It appears reasonable to expect that phosphates encapsulated in plastic would be extremely effective because slow release would produce high concentrations of insecticide over a long period of time. This possibility will be investigated in the Department for the control of insects during storage of dried fruit and tree nuts. Analytical methods are not now available for measuring plastic-encapsulated insecticides without interference from the plastic. In order to pursue this promising area of research, it is essential that rapid, simple, economical procedures for encapsulated insecticides be developed for analysis of laboratory formulations and residues on food commodities.

**d. Application in Foams.**—In recent months, much interest has been shown in the possible use of foams as carriers for agricultural chemicals. In our research programs on the navel orange worm, preliminary experiments have indicated that foams have great possibilities as carriers for insecticides. Owing to the foams' ability to hang on to the tree and nuts in almond orchards, insecticides would have a longer period of penetration. In studies to date, methyl parathion, baygon, gardona, and fundal have been infused into foams.

There is no methodology in existence that is applicable to insecticides in foams. To date, in our studies on extraction and partitioning methods, we have obtained inconsistent and incomplete recoveries of samples. In order to continue studies on foams as carriers, we need analytical procedures for both foam formulations and residues on commodities after application.

**e. Application in Sprays.**—In this area, we are concerned about uniform application or adsorption of insecticides or repellents onto fabrics, packaging materials, and surfaces of storage facilities. It is extremely difficult to correlate analytical residue data with time studies and effectiveness of pesticide treatments. Most likely, surface phenomenon are involved. The alkalinity of some spots on the surface may degrade the compound applied. Soluble dirt and stains on fabrics or cellulose may repel the application. Active sites may attract the pesticide. In any case, the application is not as uniform as might be expected from the technique of treatment. A study is needed to learn how and why these compounds are adsorbed or repelled by surfaces such as animal fibers, cellulose, concrete, and paint. Such a study would benefit from a more rapid, simpler, more accurate, or more economical analytical method.

#### 4. *Structural Basis of Herbicide Action*

Intracellular target systems are being identified for at least some herbicides such as acylanilides, *s*-triazines, and substituted uracils which might be involved in the expression of phytotoxicity. For example, these herbicides interfere with electron transport and phosphorylation in isolated chloroplasts and mitochondria. The end result of this interference is inhibition of adenosine triphosphate (ATP) production. ATP energy is required to drive all cellular activity. Without energy, cellular functions and growth are arrested. Hence, for the first time, the inhibitory action of a large group of herbicides can be studied *in vitro* on processes that conceivably are related directly to their *in vivo* toxic action. Up to this time, the studies have been primarily descriptive. Attempts are being made to elucidate the chemistry of the interaction, the bond relations between the inhibitor and the affected site, and the conformation assumed by the inhibitor at the target site.

Inhibitors must possess properties that will enable penetration to the target site and assumption of the precise spatial configuration required to complement the molecular architecture of the active center and, hence, to block the key reaction. Properties possessed by the inhibitors which could conceivably be of importance include partitioning characteristics (hydrophilic/lipophilic balance), steric relations (the ultimate attainable configuration), resonance, keto-enol tautomerization, *cis* or *trans* relation of the amide hydrogen and the carbonyl oxygen, and the possession of a critical charge by particular substituent groups which participate in intermolecular interactions at the active centers. Molecular orbital calculations should indicate sites of probable reactivity on the herbicide molecule. However, of the one attempt that has been published in which electron densities were related to inhibitory activity, for a series of acylanilides, informative results were not obtained.

Very little information is available relative to the forces that are involved with the interaction of herbicides and active sites. Evidence obtained to date indicates that formation of covalent bonds is not involved; instead the bonds are much weaker. The forces that might be involved such as hydrogen bonds, nonpolar dipol-dipol interactions, and van der Waals forces remain to be identified.

To date, a few of the chemical and physical characteristics of the interaction outlined above have been elucidated. For the most part, physiologists have attempted to obtain some of these answers with only limited assistance from analytical chemists. Conceivably, answers could

be obtained more effectively with the assistance of highly skilled and interested chemists. While the implications of the elucidations at this time are of fundamental interest, they could have real and practical importance in designing and developing more specific and highly selective herbicides. If the chemists charged with synthesizing new herbicides can be given data relative to the molecular architecture of active sites, and the chemical and physical properties that are required for inhibition of the target system, it should be possible to tailor-make herbicides.

### E. NITROSAMINES

In 1956, Magee and Barnes [29] demonstrated that dimethylnitrosamine was carcinogenic, initiating a considerable amount of research on this class of compounds. Initially, the pharmacological activity was the important aspect but, since about 1962, the presence of nitrosamines, primarily dimethyl and diethyl, in a variety of food substances has been reported. Since this becomes a matter of prime importance to public health, the quantitative determination of nitrosamines in foods and other agricultural products assumes great current significance.

Two major techniques for determining microquantities of these compounds have been reported: (1) TLC and (2) GLC.

TLC methods developed by Preussman *et al.* [30] and by Sen *et al.* [31] involved separation on Silica Gel G by several solvent systems. Both techniques require the use of duplicate spots and both methods depend on photolysis of the *N*-nitroso linkage for the identification reactions. Both techniques may be subject to interference due to artifacts extracted from foods (*e.g.*, substances such as phenols, fatty acids, pigments, and amines). Sensitivity of this method is about 0.3 to 0.6  $\mu\text{g}$  of nitrosamine.

GLC has only recently been used for the quantitative determination of nitrosamines. Two procedures, one developed by FDA [32] and the other by the Department of Agriculture [33], involve the use of the Alkali Flame Ionization Detector (AFID) which is specific for nitrogen compounds containing two nitrogen atoms. The mechanism of action of the AFID is unknown. Sensitivity to nitrogen compounds is about the same as the normal Flame Ionization Detector, but sensitivity to hydrocarbons is reduced by a factor of 10,000 which results in the selectivity. About 5 ng of dimethylnitrosamine can be detected by this method.



Regardless of whether TLC or GLC is used, the major problem in nitrosamine measurement at present is the isolation and cleanup procedure. Nitrosamines vary widely in their characteristics and do not respond uniformly to recovery procedures. The cleanup factor is important because there are many food components that interfere with measurement of nitrosamines.

With improved methods of obtaining relatively clean samples of nitrosamines from food products, adequate procedures for determining concentrations quantitatively will be no problem.

### XIII. Measurement of Pollutants

Air pollutants that have been reported to affect agriculture [34] are of two major categories: particulate and gaseous [35]. Particulates can be described as being inorganic or organic in nature. Dusts derived from construction sites, feedlots, and cement facilities are referred to as inorganic, while particles such as polynuclear aromatic hydrocarbons are organic particles. Inorganic gases are carbon monoxide, carbon dioxide, sulfur oxides, hydrogen sulfide, nitrogen oxides, ozone, and hydrogen fluoride. Organic gases and vapors are hydrocarbons, aldehydes, alkyl nitrates, organic peroxides, tetra alkyl lead, pesticides, and methyl substituted sulfides. Figure 5 shows damage to a tobacco leaf caused by photochemically generated oxidants.

Air pollutants are monitored by one or a combination of the following methods: membranes, molecular sieves, atomic absorption, ultraviolet, or infrared spectroscopy; or TLC, column chromatography, or GLC; or titrimetric or conductivity measurements.

Inorganic particles are identified by light or electron microscopy, characterized by x-ray diffraction, or by helium-neon laser, and the density in the atmosphere is determined by integrating nephelometers. Their chemical composition is determined by atomic absorption spectroscopy. Organic particles are collected on membranes, separated by chromatography, and characterized by ultraviolet, infrared, or fluorescence spectroscopy. Unless complete separation of the air components is achieved by chromatography, the spectral analyses have been found to be incorrect.

Carbon monoxide at 0.05 to 2 ppm is measured by GLC, but hydrogen, olefins, and aldehydes interfere. Carbon dioxide can be measured by



Figure 5. Tobacco leaf damaged by oxidant air pollution.

GLC and mass spectroscopy with an accuracy of 1 percent in the presence of nitrogen or air. Infrared  $\text{CO}_2$  gas analyzers are also used, especially in laboratory studies. Sulfur dioxide is measured by conductivity or colorimetrically by West-Gaeke, but air contaminants ( $\text{HCl}$ ,  $\text{NH}_4$ , and chlorine) interfere. A long path spectrophotometer with fine structured bands ( $3,000 \text{ \AA}$  region) measures low concentrations of  $\text{SO}_2$  without

interference. The loss of reflectance from a silver membrane surface is a sensitive method for measuring hydrogen sulfide. Nitrogen oxides and ozone do not interfere.

Nitrogen oxides can be identified by concentrating them on Molecular Sieve 5A and releasing them at 250 to 300 °C for GLC. Sensitivity is 0.19 ppm. Nitrogen dioxide is also measured coulometrically, but little is known about interfering substances. Ozone is generally measured by Mast Ozone Meters which measure the current imposed against a 0.24 V circuit from the oxidation of potassium iodide to iodine. Nitrogen oxides and peroxy compounds interfere with the measurement. Long path infrared can distinguish ozone from other materials, but the cost renders it useless for continuous analyses. Hydrogen fluoride is measured by GLC.

Hydrocarbons ( $C_2 - C_{12}$ ) are identified by GLC, using polyethylene glycol or squalene columns. Aldehydes are found to be present by either trapping them in bisulfite solutions or reacting them with dimedone for fluorimetric determinations. They can be derivatized and separated by GLC. Alkyl nitrates are characterized by GLC on polyethylene glycol at ambient temperatures. Organic peroxides are identified by either colorimetric or ultraviolet procedures.

Tetra alkyl lead is trapped on activated charcoal and reacted with dithizone in air samples of 100 to 300 m<sup>3</sup>, or by GLC with a range of 3 to 400 ng/m<sup>3</sup> and 95 to 99 percent accuracy. Methylated sulfides are identified by direct GLC.

Since the chemical diversity of constituents in the air is so great (3 to 4,000 identified), the challenge to the analytical chemist and the manufacturer is to develop instrumentation that is reasonable in cost, that is easily operated and requires little attention in the field, that can employ analytical means for identifying several air components in a region of the country, but with the added capability of screening out all but one component at a time. The instrumentation must be portable and have the sensitivity to be useful in field conditions.

We are concerned not only with pollutants that affect agriculture, but also with pollutants that are caused by agriculture. For example, in connection with management of feedlots we need improved methods for determining volatile nitrogen-containing compounds. Figure 6 shows a feedlot on which management systems are being developed for limiting pollution of streams and ground water.

Results of ground water studies conducted below and near feedlots in Colorado and Nebraska suggest that minimum pollution occurs if anaerobic conditions prevail below the feedlot surface. However, air sampling





Figure 6. General view of a beef cattle feedlot.

measurements show that appreciable amounts of ammonia and odors are generated under these conditions.

In air sampling equipment placed at various locations in or near feedlots, about 20 percent of the trapped nitrogen compounds were not ammonia. Qualitative tests indicate that these compounds are amines, fatty acids, and indoles. However, standard laboratory techniques fail to recover sufficient quantities of these compounds to express them quantitatively. If feedlots are to be managed to avoid air and water pollution, a quantitative measurement is needed. Current methods are most inadequate for developing management practices for minimum odor and ammonia pollution.

Another area of concern is that of pollution caused by food industries. Research is underway in many areas of agriculture to develop methods for abating pollution from specific agricultural activities. One of the areas of emphasis is the reduction of pollution in poultry processing plant effluents. Researchers are charged with developing processes and techniques that will reduce waste loading in poultry processing plant effluents. One of the most important problems in this area of research is the



lack of a single, accurate, rapid method to measure the strength of organic wastes in effluents. BOD is the test most commonly used. There is no measure of the accuracy of the BOD test on wastewater. It is possibly more of an art than a science. Even though it is a relatively simple technique, its accuracy depends on choosing the right "seed." Samples cannot be collected and stored before analysis; and single samples are almost impossible to analyze since an error in seed choice would void the sample analysis, but the error would not be known until 5 days later.

Several other tests are in use today which in some way relate to or can be related to BOD for specific operations, but they are not acceptable as a replacement for BOD. Chemical oxygen demand (*i.e.*, reduction of oxidizing agent) is a 2-1/2 hour test and, in general, does not correlate well with the BOD test. Total organic carbon can be determined in 2 or 3 minutes and has been strongly advocated by some because of its speed. Suspended solids, volatile solids, and other factors are measured but, to date, none of these measurements replaces BOD. Both in research and plant scale evaluations, a test is needed that can be performed quickly and which will give an accurate measure of the effect that a waste material will have on a natural stream. Preferably, the method should also be capable of monitoring the available oxygen content of rivers, lakes, and streams continuously.

#### XIV. Molecular Properties of Natural Polymers

In this area, there is need for conformational analysis of fibrous proteins to identify the amino acid residues important to the general structure of the protein. According to current views of molecular biology, the primary structure of the protein (the sequence of individual amino acid residues in the chain) determines its secondary and tertiary structure; namely, how the protein chain turns, twists, and folds upon itself. The physical and chemical properties of the protein depend almost entirely on the topological identity of the amino acid residues; for example, the groups that are exposed, the shape of the reacting site, and the residues that form the reacting site. Some amino acids are more important to the structure than others either because of their type, or their specific location in the chain, or both. Substitution, addition, or deletion at these critical areas will profoundly affect the macromolecular protein structure and function. If the intimate details of these composition-structure-functions relationships were known, the chemical and physical properties of proteins could be manipulated at will.

At present, there are no straightforward means for determining the configuration of individual atoms within the chain of fibrous proteins. Globular proteins, on the other hand, since they can be obtained in pure crystalline modifications, are amenable to precise structural determinations by means of x-ray diffraction techniques. Fibrous proteins, such as collagen, the keratins, and silk, because of the absence of a submicroscopic identically repeating structural unit, are not subject to detailed x-ray analysis. The kinds of conformation presently obtainable relate to their macroscopic properties; molecular information is almost entirely inferential and incomplete.

Two routes offer promise for solving this problem. Scanning electron microscopy of very high resolution may be able to identify the overall chain conformations and the location of some specific amino acid residues. Armed with sequence analysis data, one might be able to piece together a fair picture of the protein structure. Another fruitful avenue of research appears to be in certain branches of spectroscopy. With sufficiently high resolution and sensitivity, it would be possible to assign specific energy transitions to given amino acid residues in the protein. By observing the change in these transitions as the protein folds, unfolds, and interacts with various solvents, it may be possible to establish the role played by many of the amino acids in the structure. NMR and Raman spectroscopy, with the best instrumentation, appear to have developed to the point where studies of this nature are becoming feasible; as these techniques improve, unusual opportunities will exist in these areas.

Similar to this problem of conformational analysis of fibrous proteins is the problem of relating surface properties to polymer composition. In recent major oil spills, the relatively small portion of contaminated birds that survived decontamination efforts is perhaps a dramatic indirect reflection of how incomplete is our understanding of surface properties of natural polymeric materials such as wool, mohair, and feather keratin. Even though the overall composition of these materials is proteinaceous and tends to be relatively hydrophilic, the nature of the surface layer is such that these materials display hydrophobicity to bulk water. As yet there is only indirect evidence that these surfaces may be comprised largely of methyl or methylene groups which mask the hydrophilic nature of a polyamide (protein) structure. The identification of organic groups at an air/solid interface continues to be a problem of importance in characterizing surfaces more thoroughly in our search for understanding biological systems and in defining adhesion and wetting phenomena. Some of the more important methods which have been used with some success include Multiple Internal Reflection Spectroscopy (MIRS), Electron Spectroscopy

py for Chemical Analysis (ESCA), and surface wetting behavior (*e.g.*, contact angle determinations).

MIRS is limited in usefulness in that the depth of penetration of the reflected energy is more than the first molecular layer so that the spectra obtained do not solely represent the constitution at the air/surface interface. ESCA is a promising, relatively new technique which is useful for analyzing up to the first 100 Å of a surface. The technique is sensitive to the oxidation state of the element and chemical environment of the atom. At present, it has greater utility in the detection of noncarbon atoms, but improved resolution may change this situation. Measurement of wetting behavior of the surface is an indirect method, but one of the most sensitive in detecting changes in surface composition. The difficulty, of course, lies in correlating wetting changes with actual changes in composition. Additional methods for determining the chemical nature of surfaces are clearly needed.

The third problem in the area of molecular properties is related to the evaluation of intramolecular heterogeneity of collagens. Much confusion and uncertainty in collagen chemistry arises from the fact that while a given collagen preparation made from the tissue of a vertebrate animal gives a consistent physical characterization, preparations from different individuals of the same species display a wide variation in physical characteristics. These differences seem to exist at all four levels of molecular organization. Since it is very likely that function and viability of a given tissue is determined by all of its components, and collagen is invariably one of them, a study of the structure of collagen in a given tissue and how it correlates with the nutrition, development, and character of that tissue seems important.

One application of such a study would be to correlate the structure of collagen in meat with the various qualities sought by the consumer, such as flavor and tenderness. Quality could in turn be related to the nutrition and environment of the meat animal during its developmental phase.

At the present time, the only work of this nature being carried out is in the field of human geriatrics. Progress is slow because effort is limited to studies of amino acid distribution and sequence. A more fruitful approach seems to be the use of electric birefringence, which provides an extremely sensitive technique for measuring subtle differences between molecules.

Our final problem in this area is that of determining molecular weight in the 1,000 to 15,000 range. The classical methods for determining molecular weights below 500 (namely, the ebullioscopic and cryoscopic methods) have largely been replaced by vapor phase osmometry. This thermoelectric vapor-pressure method has been shown to be applicable

for molecular weights below 500 to 1,000 providing the correct solvent and standard are used.

In the case of larger molecules, light scattering for weight average molecular weights and membrane osmometry for number average molecular weights are applicable to materials as low as 10,000 to 20,000 if considerable care is exercised in the measurements. This leaves an area of roughly 1,000 to 15,000 for which no really reliable method is available. The problem is to find a suitable new method for this range or possibly to extend the range of an existing method such as vapor phase osmometry. The American Society for Testing and Materials and the AOAC are both studying this problem with the hope of establishing an official method for determining molecular weights in this range. Any suggestions that would lead to a solution of the problem would be greatly appreciated by both groups.

## XV. Automation and Instrumentation

Automation and instrumentation present the analyst with both opportunities and problems. We would like to mention some problems and opportunities related to (1) computer-aided analytical chemistry, (2) instrumentation suitable for increasingly smaller quantities of materials, (3) increasing the resolution of electron microscopy, (4) developing low-temperature ashing procedures, and (5) computer control of vegetable oil refining.

Automated analysis is capable of producing more data at a faster rate than can be interpreted [36]. Mountains of recorder paper from our GLC are forming in each of our laboratories. The answer lies in more assistance from the computer, which can be programmed to present data in graphical form ready for human interpretation.

Computer-aided analytical chemistry is not limited to the acquisition and processing of experimental data, but it also opens the door to more efficient analytical data management. Through computer control of sample flow through the laboratory, the supervisor is assured that samples will not be lost, the analyst knows the backlog of work that faces him and how much has been done, and the recipient of analytical data can receive an immediate update of all analytical information relating to the samples of his interest.

Computer-guided analysis enables us to make instant comparison between theory and experimental results. This approach contrasts with the conventional manner of waiting for days or weeks after an experiment



is completed to determine whether the range, number, and accuracy of measurements are sufficient. Thus, parameters may be varied as needed during the course of an experiment.

A major analytical problem facing the laboratory today is the need to accurately analyze increasingly smaller quantities of chemicals in animal tissues and products. To meet this requirement, it is necessary that more and more significant instruments and procedures be developed. This, in turn, requires that all chemicals used in the analytical procedure be of highest purity—in many instances, of greater purity than is commercially available. Instruments must have increasingly more specific and sensitive detection systems which will increase the complexity while decreasing flexibility. As a result, the “down time” of an instrument for repairs or to change or alter the detection systems can become a significant expense item and a major cause of lost time in the laboratory. In many cases, it is necessary to have a technician come in from a regional office or the headquarters of the company producing the instrument.

As procedures increase in sophistication and complexity, the chance of error in manipulation increases. The scientist or technician at the laboratory bench must take ever-increasing precautions to prevent contamination of the sample being analyzed and to decontaminate the equipment after use. Animal tissues and products must undergo increasingly more rigorous and complex treatment to isolate the desired chemicals for detection, thus again increasing chances of loss or error.

The localization of specific molecules within cells and subcellular components as an analytical adjunct to electron microscopy is just beginning. The contrasting and imaging of organic molecules is limited by two factors. These molecules cannot be distinguished from the carbonaceous matrix in or on which they are supported or from neighboring biological molecules. In addition, the low inertia of the atomic components make them susceptible to irradiation damage from the illuminating beam of electrons.

Both of these problems could be solved by the addition of heavy elements to specific organic molecules. The heavy elements would increase contrasting ability and because of their mass, be more stable in the electron beam.

Current methods have involved the use of metal-containing light microscopic stains or metal adducts of antibodies. Both of these techniques, although they may be very specific in their mode of action, have resolution capabilities on the order of tens of angstroms instead of an order of magnitude lower which would allow the imaging of specific molecules, not aggregates of them.

If perfected, this technique would allow for localization of mycotoxins, pesticides, antibiotics, and their metabolic derivatives. It would also facilitate analysis of native components which play a role in spoiling, textural, and color changes. The current development of high resolution analytical microprobes would, if coupled with this technique, allow the simultaneous qualitative evaluation of several metal derivative compounds on a quantitative basis.

Historically the analysis of the ash content of foods and fiber has always held a role of importance in establishing quality and function. Qualitative and quantitative analytical procedures in this area have advanced to the point where the rate controlling step of the analysis is now the reduction of samples to ash.

Thousands of burnt fingers and endless hours of waiting attest to the deficiencies inherent in the muffle furnace or the wet digestion rack. In many instances recovery experiments using volatile metal complexes have demonstrated the more serious shortcomings of classic procedures. Advances in analytical procedures call for a concurrent advance in ashing techniques.

Equipment has recently been made available which claims to reduce ashing time and trouble by use of plasmas. Heralded as "cold" ashing procedures, they remain to be more thoroughly studied and developed. Certainly temperature of the plasma stream and sample temperature cannot be equated as similar.

It is most surprising that the development of the radically conceived ashing devices employing reactive plasmas have been the subject of so little research and development. Theoretically they should already have a routine place in modern laboratories devoted to food and fiber analysis.

An opportunity that we are now pursuing in the Department is computer control of vegetable oil refining and processing. Several advances are needed to enable automatic control of the various steps involved in the continuous refining and processing of vegetable oils to achieve most economical production of consistently high quality products. The successive steps involved are alkali refining, bleaching, hydrogenation, and deodorization.

In the first of these operations, after oil has been treated with alkali solution, the resulting soapstock is separated from the oil by continuous centrifugation. To minimize losses of refined oil, a small amount of soapstock is allowed to be carried over with the oil, which is subsequently washed to remove this soapstock. When too much good oil is allowed to go with the soapstock, financial losses can exceed \$100,000 per month in refineries which process 12 or more tank cars of oil per day. Needed is a

method for continuously monitoring the sodium content so that the oil loss in the soapstock and the sodium remaining in the oil are minimized. Perhaps atomic absorption analysis could be adapted for this purpose.

The bleaching operation involves treatment with a bleaching clay at high temperature to remove objectionable color. With soybean oil, in particular, chlorophyll must be removed completely from the oil. Probably an absorption spectrometric method could be adapted for continuous monitoring of color of the bleached oil.

When oils are hydrogenated, the process is monitored relatively effectively by use of continuous refractometry, but this gives only the extent of hydrogenation, not the amount of isomerization of *cis* to *trans* double bonds. Too much isomerization raises the melting point of the fat and causes excessive loss during subsequent winterization. A method for continuous monitoring of *cis/trans* isomer composition is needed, based possibly on infrared or Raman spectroscopy. This could be used to control hydrogenation conditions to minimize isomerization.

Deodorization is accomplished by steam stripping at high temperature under vacuum. Currently there is no satisfactory way to determine when this process is complete, so all oils are deodorized for a certain "safe" period of time. Deodorizer throughput could easily be doubled if instrumentation were developed to allow the oil to be carried through as soon as the process is complete.

## XVI. Summary

Along with the far-ranging applications of analytical chemistry in agriculture come many unsolved analytical problems. In fact, few agricultural scientists will admit complete satisfaction with any of their current methodologies. For some of our problems, there appears to be no current solution. These difficulties will challenge the ingenuity and creativity of analytical chemists in the future.

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## PANEL DISCUSSION

### Analytical Problems in Agricultural Science



#### CHAIRMAN

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George W. Irving, Jr., Administrator, Agricultural Research Service (Speaker)

**Firestone** — It is most important to develop accurate and rapid methods to analyze foods, plants and raw materials. It is particularly important that methods be developed that can be used in regulatory laboratories at the state and city level, that are not extremely sophisticated nor require highly specialized equipment. The problem in these laboratories is not one of total analysis but generally of analysis for a specific component, and scientists developing procedures should keep that principle in mind. Many of the methods that are available today are rooted in tradition and therefore are slow to change.

In many areas of agricultural chemical analysis, extreme accuracy is not what is demanded. An example is the protein or fatty acid profile of various foods. These profiles vary because we are dealing with natural products. However, this should not imply lack of interest in, or neglect of, accuracy. Much of the problem in the applicability of routine methods in the laboratory is really concerned with the availability of standards. The instrumentation is and has been available, but it is only recently that reference standards, supplied by commercial laboratories, have been available for fatty acid profiles. These standards can be used either internally or used without a sample to check on detector response. They are important for doing really accurate work in an absolute sense.

An example of the problems in obtaining standards for these methods is in the area of determining cyclopropenoids. These fatty acids, characteristic of cottonseed oils, are highly reactive and occur in fatty acid profiles in trace amounts. The Halphen reaction, a highly sensitive and specific colorimetric reaction, can be used to detect cyclopropenoids in biological tissue and can be used to determine that cottonseed oil is present in a mixture of other oils. These standards are difficult to prepare and have to be handled so that they will not be oxidized. The individual using this kind of methodology therefore has to prepare his own standards, which is a fairly tedious procedure, or use secondary standardization methods.

Because of the demand of the medical profession to label foods as to their fatty acid concentration, the analysis for fatty acid profile has become much more important. This has prompted some foreign countries (*e.g.* Canada) to develop an enzyme procedure for determining cis-polyunsaturates. This method has not yet been studied collaboratively but is currently under study jointly by the United States and other countries. The procedure has the advantage that polyunsaturates can be determined rapidly. This is one example of the use of enzymes to provide sensitive and specific methods.

It is also important that sensitive methods be available for the deter-



mination of mercury, lead, and cadmium and other toxic elements in food. Problems still exist with respect to digestion and clean-up even though sensitive methodology already exists for the determination of the cited heavy metals, and more techniques are being developed. Complete digestion and clean-up of fatty foods presents a special problem. It is, of course, important when we are talking about mercury, lead and other metals to take note of the form of the element: is it ionic or is it organo-metallic? For example, in the case of mercury the toxicities of the ions are much lower than those of some of its organo-metallic compounds, *e.g.* the alkyl mercury halides.

The combination of gas chromatography and mass spectrometry (GLC-MS) holds out promise for the analysis of many contaminants in foods. An example is the chlorodibenzoparadioxins. These materials arise as contaminants in chlorophenol and develop during the manufacture of chlorophenols generally. One of the chlorodioxins, the symmetrical 2, 3, 7, 8-tetrachlorodioxin, is extremely toxic, thousands of times more toxic than the usual chlorinated pesticides that we are familiar with. Thus there is a great deal of interest in determining this compound, and its cousins, at very low levels. We find that chlorodioxins may occur in chlorophenols or in fats along with many other contaminants including the polychlorinated biphenyls referred to by Dr. Irving.

There are many compounds that fit into this general class, the toxicities of which are not completely understood. At present there really isn't a quantitative procedure, but one of the most useful analytical techniques involved the use of a GLC-MS system where fairly low levels of chlorodioxins can be determined; this system is particularly important where several compounds may have the same GLC retention time. In many cases a peak from the GLC may represent four or five non-isomeric compounds which, however, can be resolved by a mass spectrometry system because of unique fragmentation patterns. However, there are problems in interfacing the gas chromatograph and the mass spectrometer. A new and better interface is required. The solution may be to have the effluent from the GC flow directly into the ion source and to use a very large, efficient pump to eliminate carrier gas in the mass spectrometer.

The work that has been done with insect attractants is an area which should be fascinating to analytical chemists because in some instances only a few micrograms of the material have been used to describe the structure of some of these attractants. Perhaps they can be used to develop new approaches to controlling insect population and reducing the widespread use of pesticides especially the chlorinated pesticides.

**Laitinen** — The approach taken in this symposium is intriguing. It is unusual and is particularly apt to be suggestive and productive in new ideas because we are dealing not so much with analytical chemists doing their "thing," as what we ought to be doing. One point about the agriculture system which makes it unique and particularly appropriate here is the fact that we now have in universities, as well as in government and industry, two new concepts coming in terms of research. One is the systems approach to research; that is, looking at a multivariant system (and now the one termed "ecosystem" is a very popular one). The other is "interdisciplinary"; that is, bringing to bear on a problem a large number of different backgrounds.

These are extraordinarily important trends which are just in their infancy and will continue as long as we can foresee. If we look at agriculture in its relationship to this approach, we see that it is unique in the sense that it has components of all elements of the ecosystem, that is, the five major components: the air, the water, the earth, the animal and the plant. Agriculture is involved with all of these, and is of vital importance to society as well. Therefore, a real opportunity is presented to universities and to government and industry, to use the systems approach and the interdisciplinary approach in agricultural research.

One definition of the systems approach would include the various levels of systems; *i.e.*, the ecosystem first of all would consist of these five major components and the transfer of materials and interconversions between them, and the results of these transfers. We can speak of the systems approach at lower organizational levels as well. Suppose we take one part of the system, say the animal kingdom, and we consider one animal as a system. He too has inputs and outputs and distributions within his individual organs. We can then go down one more level on the organizational scale and consider one organ of this animal, say the liver, and consider its input, output, transfers, *etc.* We can then go on and on, down to the cell level, then to the membrane level, then to the liquid level. When we get to the liquid level we are talking about the molecular system and here is where chemistry really pertains.

The point is then, that at these highest organizational levels we are going to be performing research, the results of which will have the most immediate impact upon society. At the lower levels we are going to perform research that has the least immediate impact, but perhaps the greatest fundamental and long range importance.

The systems approach to a problem as complex as agriculture, or ecology in general, ought to be attacked on at least five or six organizational levels simultaneously, because you can't afford to wait to start on one

until you are ready for it. This really calls for concepts of interdisciplinary research; it is no longer possible for an analytical chemist to have a complete background before having to solve these difficult and sophisticated problems. It is necessary for him to learn to work with others who bring him that knowledge; in turn he will bring to the solution of the problem techniques that otherwise would be out of reach as far as the practitioners of other disciplines are concerned.

One of the most important concepts that is necessary in analytical chemistry is that of determining forms or species of the elements. Some of you may have noticed the editorial in the June issue of *Analytical Chemistry* which was addressed to the importance of determining species; it is important to emphasize that point over and over again. Speciation is important not only in the more or less simple things, like the availability of vitamins and proteins, but also in the complex interaction of ions, molecules, oxidation states, *etc.*, in nutrition and toxicology. The development of methods for species is an exceptionally difficult problem which must be solved.

We will probably never be happy with the analytical methods available; not now, not 20 years from now, not 100 years from now—because there is a type of evolution of an analytical method, or of science itself, which makes it impossible for us to become satisfied. Consider a problem as difficult as the aflatoxin problem that was mentioned by Dr. Irving. The first question is: Have we got *any* approach? Often the first approach utilizes the practical consequences of that particular material, but does not directly identify it. If it is a poison, we can use a biological test. If it kills something, then there it is, and we can determine somewhat, the toxicity level. This is unsatisfactory in the sense that we would like to have methods that are simpler, more accurate, more rapid and more specific.

Add together all the desirable attributes of an analytical method: sensitivity, specificity, accuracy, speed, reliability, simplicity, and economy. Then, to top it all off, we would like a method that is nondestructive so that we can test our little corn seed and still have it to plant. When are we going to have an analytical method that does all of these? Then, if we start with a method that is so universally applicable and so sensitive and so specific that you could put the sample in and immediately get an answer out, you can be sure that the next complaint would be that it is too complicated: "I can't take it in my vest pocket and go out into the field and make determinations." So you can't win this game, but that is what keeps us busy and keeps us in business.

This brings us then to the point that where is this analytical work going to come from? Certainly the work in agriculture is not going to come just



from agricultural scientists and it is the same in any other field you can name. We are going to have to call upon progress in all kinds of disciplines, including physics, biology, *etc.* This is where communication comes in. We must have mediums of exchange, like this symposium and like written publications, to transfer this information from the supplier to the user.

What about the role of universities? In the past universities have suffered from one important drawback as far as application to problems of society; *e.g.* they are disciplinarian in nature. This is, however, a very essential part of the university structure and this is the way that various disciplines, not only scientific disciplines but social sciences, humanities, *etc.*, keep their validity. At the same time, there is need for something beyond this, for something in addition to this; that is the interdisciplinary approach.

Let us take a moment to define interdisciplinary in relation to another term, multidisciplinary, which is similar and is sometimes used interchangeably. The Dean of the Graduate College at the University of Illinois, Dan Alpert, has made a very useful distinction between these two terms. The term multidisciplinary implies that a multiplicity of disciplines such as physics, chemistry, biology, and so on, are brought to bear on a problem. The various disciplines, however, keep their separate identities and are brought to bear as needed.

On the other hand, the interdisciplinary approach is a problem-focused approach in which various disciplines lose their identity to a certain extent. A biologist and a chemist and a physicist would team up together daily to focus on a problem. That doesn't mean getting together once a week for a meeting, but actually working on a problem in detail together. This is something very alien to the university campus as we have discovered in the past years as we have been trying to set up such an organization. The Agricultural Experiment Station doesn't even do it, and the reason is that the agricultural colleges are essentially discipline oriented. There are such things as departments of Soil Physics, Soil Chemistry, and even Agricultural Economics. The reason for this is clear; people do not want to lose their identity within their own disciplines.

It would be a terrible mistake for us to use the same pattern again in the environmental field. We don't need to have, nor should we have in my opinion, a Department of Environmental Chemistry, and environmental this and that. Instead we should have this *interdisciplinary* mode of attack on the environmental system. This is something the universities will have to do and yet not destroy the essential quality of their traditional disciplinary approach.



In concluding let me ask what would happen if 100 years from now, or, 1000 years from now, we were to have a summer symposium on Analytical Chemistry. What would it be like? What could it be like? One must conclude that most, if not all, of the subjects that are in this symposium would be appropriate 100 years from now. That is, we are definitely going to be dealing with agriculture, with the environment, the atmosphere, the ocean, *etc.* Mankind is going to have to deal with these problems. What would it be like as far as analytical chemistry is concerned? If you have a speaker 100 years from today talking about the human body, he might be complaining about the fact that, although we have 1100 different analyses that can be run in 30 minutes as soon as the patient is admitted to the clinic, look what is missing: we still don't know whether this particular enzyme system is particularly active in this part of the liver or that part of the liver and wouldn't it be nice to know—! And that we must have methods which are more accurate, more sensitive, cheaper, and so on, and so on, and so on.

**Merritt** — Because funds are usually limited, laboratories should be organized to achieve a multi-objective mission with a minimum configuration. This means you must get the most work done with the fewest people. This is not, of course, the ultimate solution. There is a need for more trained analytical chemists and expanded educational programs, particularly those oriented toward problem solving and meeting specific needs in analytical chemistry. In the meantime it is necessary that analytical chemists do an adequate job of selling their techniques and their relationship to relevant problems.

As an example, the U.S. Army Natick Laboratory has been involved in problems of coffee distribution to the Army in both field and garrison installations. In the past, 95 percent of this coffee has been prepared from ground beans. Because of low acceptability by the troops, instant coffee of the spray or drum-dried types has not been used. However, freeze-dried coffee that meets the necessary acceptance standards is available. This coffee can be prepared using analytical chemistry as an aid both in its development and in its quality control. When you present management with the fact that annual savings of procurement costs of over \$2,000,000 and, transportation cost savings in the world-wide distribution system of over \$8,000,000 may be realized, it is obvious that it should not be difficult to obtain \$100,000 or so for sophisticated laboratory equipment.

Another major area that must be used more to advantage by analytical chemists is that of serendipity, or spin-off. Serendipity in this context means that in developing methods of analysis, many other areas of science

may be studied other than analytical chemistry. In the area of analytical chemistry the work of the Natick analytical chemistry laboratory is probably best known for some of the studies made in attempts to develop methods of analysis for the trace amounts of volatile organic compounds in complex mixtures. Twenty-five to thirty papers have been published in the last 10 years or so on gas chromatography, mass spectrometry, *etc.*, and most of these papers have appeared in analytical journals. However, what many analytical chemists may not know is that an equal number of papers on flavor and odor studies, on mechanisms of irradiation damage in liquids and proteins, on pyrolysis in proteins, *etc.*, also have been published. These papers have appeared in journals such as the Journal of Agricultural and Food Chemistry, the Journal of Dairy Sciences, *etc.*

In general the work at Natick is a two-fold activity: development of methods on the one hand and the applications of those methods to specific problems that require relevant solutions on the other. This may be a pragmatic approach but it is based on the real-life fact that very few agencies will provide funds for laboratories to work only on the development of analytical methods without a specific application in mind.

Another point of serendipity is that methods developed for one type of analysis, for example food flavors, are often also applicable to a wide variety of other studies with hardly any change except for the material in the sample flask. An example of a problem under study at Natick which illustrates a large degree of serendipity is one that concerns one of the larger Air Force Bases overseas that is using powdered milk in the mess halls and the PX. The local water used to rehydrate the milk is polluted. The problem was discovered because the milk tasted bad. The pollutants from the water were isolated and identified. In this case they proved to be from a chemical plant up stream from the air base. Developing the means of isolating compounds from the dilute aqueous solution is primarily an analytical problem, but now a method has been proposed for a large scale purification of the water for use in large scale rehydration. This kind of serendipity can produce a lot of support for analytical methods development.

Another example is the study of the changes that occur in foods upon heating, *e.g.*, the development of flavor in coffee beans brought on by roasting. For this a combined pyrolysis-gas chromatographic-mass spectrometry analysis system has been developed to study the thermolytic decomposition of proteins using a combustion furnace. The same system with a Curie point pyrolyzer becomes an analysis system for the identification of degradation-products for synthetic polymers. If the pyrolyzer is replaced with a high energy thermal source such as a carbon arc or solar

furnace, the degradation of clothing fabrics by a simulated nuclear thermal pulse can be studied. The problems associated with the disposal of packaging materials by incineration are being studied with a laser-gas chromatograph-mass spectrometry system.

Although practical considerations may dictate that efforts be directed to specific problem area needs, it must be recognized that all investigations of material composition move forward only as analytical methods provide the means. In this context, funding agencies must take more cognizance of this fact and support the development of analytical methods *per se*.

It requires very little imagination to realize the scope of applications that a single analytical procedure might find. One of the most important problems that must be overcome is that of communication between analytical chemists and those whom they are to serve. Often other scientists are not even aware of the fact that analytical chemists can contribute to the solution of their problems. Once this communication exists analytical chemists can often solve the problems.

**Rollier** — Interdisciplinary research is becoming more and more important. The remarks of Professor Laitinen are certainly true. The solution to our problem is not to set up Departments of Ecological Chemistry or Ecological Physics or Ecological Biology, *etc.*, but to have specialists in existing departments cooperate in interdisciplinary ventures. This is certainly where the future of universities lies.

One area that Dr. Irving did not have time to go into deeply is the problem of the role of trace elements. This problem is an extremely important one for nutrition studies and for agriculture in general. The University of Pavia had a contract with the International Atomic Energy Agency to study the determination of the trace elements chromium and selenium. Both of these elements are extremely important and their determination in trace amounts is important not only in foodstuffs but also in human and animal tissues. Since that time a program has been undertaken with the U.S. Navy Medical Unit stationed in Cairo, UAR, studying some diseases due to malnutrition, in which trace amounts of selenium and chromium are being determined by activation analysis.

The problem of selenium is important because it appears possible that trace amounts of selenium, a fraction of a part per million in foods and in tissues, are persistently lower in the Mediterranean areas than they are in North America. Biologists know that the effect of selenium on growth is peculiar, especially at the 0.2-0.4 ppm level. It may be that a certain amount of selenium is essential in the diet. Selenium deficiency may be

responsible for many different things, for instance for the fact that the average height of the people in the Mediterranean area is less than that of the people on the North American continent. This is, of course, at present conjecture, but it is important to point out how essential accurate and precise determination of trace elements in human tissues, in plants and animals, and especially in foodstuffs may become in the near future. For this reason chromium and selenium should be added to Dr. Irving's list of minor elements such as iron, manganese, boron, copper, zinc, and molybdenum. Neutron activation analysis is an excellent method for determining many of these trace elements.

Determinations of trace elements in foodstuffs should be made routinely if we are to understand their role. The problem of detecting insecticide, fungicide, herbicide, pesticide, *etc.*, residues in foods and crops, is of the greatest importance. Most of these chemicals contain phosphorus and/or sulfur. Synthetic sexual lures, combined with chemical sterilizers usually contain fluorine, phosphorus, sulfur, and bromine atoms in their molecules, fluorine and phosphorus being predominant. Analysis by neutron activation makes it possible to determine phosphorus and bromine in tiny amounts of agro-chemical residues which may be in food products. There are many nuclear reactors in nearly every country of the world capable of doing these analyses.

It may be possible to tag agricultural chemicals with rarely occurring but high nuclear cross-section materials so that pesticide residues could be determined easily by activation analysis. It would, of course, be necessary to determine that correlations between pesticides and the added element were constant as a function of time. For this reason it would probably be necessary to have the tagging material an integral part of the molecule. This technique was suggested by scientists working in forensic analysis some time ago, and has been applied in many cases. The amount of tagging material being added would be very low and therefore probably non-toxic although this would have to be proven.





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## CHAPTER 5

# ANALYTICAL PROBLEMS IN AIR POLLUTION CONTROL

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The role of measuring techniques in activities ranging from research to regulatory activities of the Environmental Protection Agency will be discussed. Field and laboratory instruments and manual techniques will receive consideration. Both point-sampling and remote types of instrumentation developments will be reviewed. Chemical and physical transformations contribute to the formation of new or modified pollutant species. Air quality measurements must be capable of properly following such transformations. Especial emphasis needs to be given to the conversion of gaseous into various submicron particulate species. The mass, particle-size distribution and details of chemical composition of these particles is required. Examples will be given of new and improved types of air quality measurements, stationary source and mobile source emission measurements which may be needed to meet air quality and emission standards.

**Keywords:** Air pollution; air quality instrumentation; air quality measurements; anion analysis; Clean Air Act; elemental analysis; measurement of atmospheric gases and vapors; measurement of atmospheric particles.

## I. Introduction

A small program concerned with development of new or improved techniques for measuring air pollution has existed for quite some time, but particularly from the inception of Federal activities in 1955. Until 1967, this program was primarily concerned with support of research and monitoring needs.

Between 1955 and 1967, a large number of laboratory analytical techniques were developed for air pollutants. Considerable research was done on development of colorimetric and chromatographic techniques. These techniques were applied to air quality measurement and to motor vehicle emissions research. During this period a small number of air quality instruments, particularly those for oxidants and hydrocarbons, were evaluated and improved. Most of the colorimetric procedures used in air quality instruments were developed or improved during this period.

By the mid 1960's, it was apparent that new techniques and sensors were becoming available. Gas chromatographic techniques were well enough developed to justify their use in monitoring instrumentation, but the necessary resources were not available. The costs involved in incorporating a laboratory technique into an effective monitoring instrument for routine general use are considerable.

The Clean Air Act, as amended in 1967, referred to instrumentation requirements in two sections. Section 104 states: "The Administrator may conduct and accelerate R/D of low cost instrumentation techniques to facilitate determination of quantity and quality of air pollution emissions, including, but not limited to, automotive emissions." In Section 133, annual reports to Congress are required, including a report of progress on "The development of quantitative and qualitative instrumentation to monitor emissions and air quality." These passages in the Act stimulated for the first time the allocation of resources for instrumentation R/D. The pace of these activities has accelerated slowly but significantly since the passage of these 1967 Amendments.

It was clear by 1967 that most of the instruments available for measurement of air quality were deficient in sensitivity, specificity, or ease of operation and maintenance. Emphasis was placed on the development of a new generation of instruments using physical principles rather than wet chemistry. Many of these instruments can be used, with some modification, for monitoring emissions from motor vehicles and from stationary sources.

The 1970 Clean Air Act also involves additional measurement capability for mobile emission sources. The development of new power sources or propulsion systems requires that we have analytical procedures capable of qualitatively and quantitatively measuring pollutants "which cause or contribute, or are likely to cause or contribute, to air pollution which endangers the public health or welfare but for which standards have not been prescribed" in accordance with Section 202 of the 1970 Clean Air Act. In general, Section 202 requires that measurement techniques be



prescribed for any air pollutant, for which standards are promulgated, that is emitted from motor vehicles.

Evaluation of instrumentation for measuring emissions from stationary sources has recently been initiated in accordance with provisions of the 1970 Clean Air Act. Section 110 (2F), on implementation plans, states that approval of such plans shall include, among other aspects, "requirements for installation of equipment by owner or operators of stationary sources to monitor emission." Section 114, on inspection, monitoring, and entry, states: "The Administrator may require the owner or operator in carrying out provisions of Section 110, 111, and 303 to (C) install, use and maintain monitoring equipment or methods; (D) sample such emissions (in accordance with such methods, of such locations, at such intervals, and in such manner as the Administrator shall prescribe)." These provisions certainly will require the acceleration of both R/D and the evaluation and standardization of instrumentation and of sampling and analysis procedures for emissions from stationary sources.

A list of important areas of need for air pollution measurement capability are listed in Table 1. The listing is divided among air quality, motor vehicle emission, and stationary source requirements. The order within each area proceeds from research on pollutant composition and concentration, as related to atmospheric characteristics and biological effects, to regulatory needs. Most of the techniques presently available resulted

Table 1. Air pollution program elements requiring instrumentation and measurements development, evaluation, and standardization.

- Air quality surveillance networks
- Atmospheric and plume research investigations
- Biological effects field investigations
- Air quality standards
  
- Motor vehicle emission composition investigations
- Motor vehicle certification and production line testing
- Surveillance of production vehicles with mileage accumulation
  
- Investigation of stationary source emissions composition
- Stationary source emission standards
- Determination of compliance with emission standards

from earlier R/D programs of air quality surveillance activities. However, the Environmental Protection Agency will soon outrun this reservoir of measurement capability when it begins impending regulatory programs. It is essential that measuring requirements for R/D projects be supported now, or the measuring techniques will not be available for regulatory requirements later. The complexity of the process and the timing involved will be considered in greater detail later in this discussion.

Important criteria for air quality instrumentation are listed in Table 2. Of course, these same criteria apply to many other instruments. Sensitivity requirements can vary greatly. For monitoring air pollution, the ability to measure 10 to 1000  $\mu\text{g}/\text{m}^3$  is required. It appears that at stationary source applications the concentration range will be 100 to 1000 times higher. It follows that an instrument may have more than satisfactory sensitivity for monitoring stationary source emissions, but totally inadequate sensitivity for measuring ambient air quality. Response times of 1 second or less are needed for some motor vehicle applications, whereas periodic analyses once every 5 or 10 minutes, or even less frequently, are required for other air pollution measurements. Fortunately, it is possible to develop instruments with appropriate characteristics for the entire range of applications.

Table 2. Criteria for air monitoring instrumentation.

- Compatibility with sampling system
- Sensitivity sufficient to meet monitoring application
- Specificity of response to pollutant of interest
- Adequacy of response time for application
- Simplicity of construction and operation
- Reliability and reproducibility of operation
- Compatibility with data handling equipment

A reasonable position is to develop the minimum set of different instruments. Such an approach will tend to minimize instrument development costs, reduce problems of reliable commercialization of instrumentation, and speed standardization and collaborative testing programs. This approach must also produce instruments adequate for each of the applications listed in Table 1 at a reasonable cost per instrument.

A sequence of activities required to develop and commercialize an instrument is given in Figure 1. In many past and some present instrument

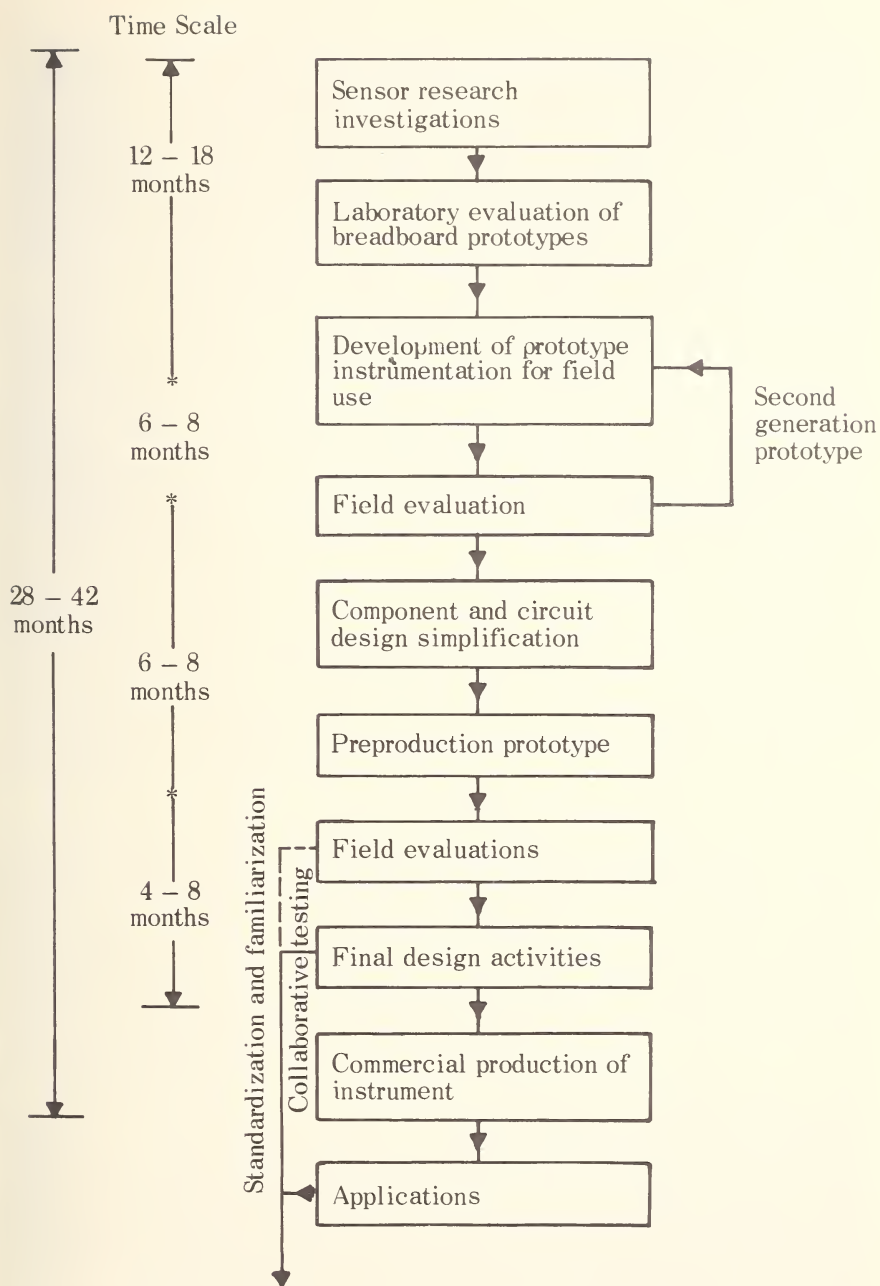


Figure 1. Sequence of activities in developing, evaluating and standardizing air pollution monitoring instrumentation.

projects, a pronounced tendency to bypass a number of steps in the given process has prevailed. The net result has been either unusable instruments or expenditure of much work in rebuilding instruments. Aside from the cost and inefficiency involved in them, such abbreviated approaches actually prove more time consuming than an approach that systematically follows through on the necessary steps as listed. As indicated in the time scale, it is most unusual for this sequence to take less than 2 to 2-1/4 years; the sequence can require more than 3 years if research problems occur early in the process or if prototypes must be redesigned.

The substantial period of time indicated here as essential for development of a satisfactory instrument reemphasizes the need to anticipate instrument requirements very much in advance of regulatory deadlines. Unless they are developed for R/D projects, prototype instruments will not be available when required for regulatory standards. Cumbersome and often inadequate manual analytical methods must be substituted, to the discomfort of all, while the overdue instrumentation is being developed.

## II. Air Quality Measurements

### A. INSTRUMENTATION FOR MEASURING ATMOSPHERIC GASES AND VAPORS

This discussion will be concerned primarily with the present status of and anticipated needs for instruments that can monitor ambient air. Point sampling instrumentation, along with remote and long-path-type instrumentation, will be considered for the monitoring of gases. Ambient air monitoring needs will be considered to include not only determination of urban pollution, but measurement of pollution on a regional and global scale. Because a number of atmospheric substances of concern do not result from primary emissions, but from chemical reactions, atmospheric transformations must be considered.

Methods of analysis and instrumentation available up to about 1966 have received detailed consideration in a number of reviews [1-7]. Therefore, this presentation will emphasize advances in the last several years. Our first concern is with the justification for the development of new or improved instrumentation. It will be necessary to consider the deficiencies of instruments to analyze ambient air that have been available until recently.



Almost all of the air-monitoring instruments installed up to 1970 were based on analytical approaches developed 10 to 15 years earlier. Many of the individual types of instruments are cumbersome, of low specificity, of limited sensitivity, and difficult to maintain because of complexities of design. Some of the instruments suffer from only one or two deficiencies, while others fail in almost all aspects. As a result, the amount of valid measurement data obtained has frequently been limited.

Attempts were made to improve some of these instruments with respect to specificity of response for ozone [8] and hydrocarbons [9,10]. Such activities were viewed as temporary expedients necessary because of the lack of funds to develop new instruments. The approach usually taken was to utilize a substrate in the inlet system of the instrument which would remove or hold back interfering substances. Experience has shown that such systems do not work very effectively in routine monitoring operations, although they can be handled successfully by R/D personnel in field studies. No R/D activity can anticipate all of the problems associated with operations under a variety of routine conditions in the field. Variability in time to partial or complete breakthrough of the interferences through the substrate under varying atmospheric conditions is one of the problems often experienced. In general, instruments that require use of auxiliary clean-up systems to achieve specificity are more prone to give incorrect results than instruments whose basic sensing principles confer the required specificity.

A number of instruments have been in common use in monitoring networks during the past 10 to 15 years. When these instruments are considered in terms of sensitivity, specificity of response, simplicity of construction and operation, or reliability and reproducibility of operation, they have proved inadequate, usually with respect to several of these criteria. As a result, few concerned persons have expressed satisfaction with instruments available during the last 10 or 15 years in monitoring networks. Unfortunately, little incentive and fewer resources were available to remedy the situation until recently.

Although the resources available in the last several years have been modest, considerable progress has been made, particularly with respect to new or improved instruments for measuring inorganic gases. Instruments have been developed which are now receiving or have received field evaluation for sulfur oxides, ozone, carbon monoxide, nitric oxide, and nitrogen dioxide. In addition, a better technique for determination of non-methane hydrocarbon has been developed. Most of these instrument developments have utilized sensors or laboratory equipment produced by research in recent years. However, a considerable range of activities has

been required to convert these into usable air pollution monitoring instruments. Sensors had to be evaluated with respect to sensitivity and specificity for urban air pollution requirements. Long-term stability and reproducibility or operation had to be evaluated. Breadboard laboratory equipment had to be packaged as field instruments. Field studies have been required to permit comparisons of instruments used for each of the several pollutants under representative ambient air conditions. Such investigations, from laboratory evaluation of the potential for applicability of a sensor to air pollution needs through field evaluation, require several years of continuing efforts. Field evaluations indicate that all of these instruments have the sensitivity and specificity needed for urban air pollution applications. The instruments also should be capable for measuring so-called near-urban pollution.

The gas chromatographic technique for carbon monoxide and methane utilized in former years in laboratory photochemical studies [11-13] was developed into a convenient monitoring instrument [14,15]. In combination with a capability for measuring total hydrocarbons, such a gas chromatographic analyzer provides a highly specific and sensitive means of analyzing carbon monoxide and nonmethane hydrocarbons over a wide range of atmospheric concentrations. As a carbon monoxide analyzer, the gas chromatograph is much more sensitive and specific than current non-dispersive carbon monoxide analyzers. This approach can also be extended to include monitoring for other gaseous hydrocarbons. The direct analysis for methane is much more desirable than the earlier attempt to use substrates to remove hydrocarbons other than methane [9,10]. This type of technique has given erratic results in routine monitoring activities because of the care necessary to maintain the characteristics of the substrate utilized to provide the specificity required. Another technique developed for methane and other hydrocarbons involves selective combustion, with subsequent detection by a water sorption sensor [16].

The present oxidant analyzers are unsatisfactory monitoring tools. The "oxidant" has no exact meaning since the response obtained depends on the presence of various interfering substances. The substrate used to eliminate sulfur dioxide interference oxidizes nitric oxide to nitrogen dioxide and thus increases the signal caused by nitrogen dioxide [8]. Recently, it has been shown that hydrogen peroxide is an atmospheric oxidant, but the response of commercial analyzers to this oxidant is poor [17]. It should be evident that each of the major oxidants should be measured separately. Several ozone analyzers have been developed and evaluated under field conditions. All of these instruments are based on utilization of chemiluminescent reactions [18]. The three types of reactions in-

volved are as follows: (1) reaction of ozone with Rhodamine B adsorbed on silica-gel disc (Regener Method), with emission at 0.59 micrometer; (2) mixing of ozone with excess nitric oxide, resulting in chemiluminescence from excited nitrogen dioxide that extends from 0.6 to 3.0 micrometers and (3) mixing of ozone with excess ethylene, resulting in a chemiluminescence peak near 0.43 micrometer (Nederbragt's Method). All of these systems provide a specific and sensitive means of analyzing ozone rapidly over an adequate linear range. However, both the Regener [19-21] and the ozone-nitric oxide techniques [18,22] have several disadvantages, compared to the Nederbragt technique, for use in a routine air monitoring instrument [18,23,24].

The Regener approach requires preparation and calibration of the disc. Because of the activation and decay characteristics of the chemiluminescent surface of the disc, a 4-minute mode of operation is preferable; the mode involves a calibration cycle, purge cycle, sample cycle, and purge cycle [20,21]. This type of operation also complicates methods for obtaining signal readout. The ozone-nitric oxide reaction requires use of reduced pressure to avoid quenching the chemiluminescent reaction [18,22]. Detection of emissions in the spectral region involved dictates the use of photomultipliers near the edge of the chemiluminescent response above 0.6 micrometers; thus cooling to  $-20^{\circ}\text{C}$  is required to obtain a sensitivity down to 0.01 ppm. The Nederbragt-type detector can operate at atmospheric pressure without quenching and with a sensitivity down to 0.003 ppm at  $5^{\circ}\text{C}$  with a less expensive photomultiplier [18,23,24]. The ozone-ethylene reaction can be used with a detector of less complicated design, excellent operation, and good cost and size characteristics.

The flame photometric detector has received considerable attention in our program for measurement of sulfur dioxide, hydrogen sulfide, and other sulfur compounds [14,25,26]. This technique involves a response to both inorganic and organic sulfur compounds because these substances form  $\text{S}_2$  species in the flame zone which are responsible for the emission observed. Several field evaluation studies already completed have demonstrated the effectiveness of this sensor, both as a sulfur analyzer and as a detector in a monitoring gas chromatograph [26]. This approach is more attractive than most of the other techniques available for measuring sulfur compounds. The flame photometric sensor has the following characteristics: (1) high specificity for sulfur compounds if emission is measured between 0.39 and 0.40 micrometers; (2) high sensitivity (0.005 ppm); (3) linear response in ambient air concentration range; (4) fast response characteristics; (5) and a gas flow system (no liquid reagents). The flame



photometric sulfur detector is also the only detector sensitive to sulfur compounds that can be used practicably in a gas chromatographic analyzer. When sulfur dioxide is the predominant sulfur species present, the flame photometric analyzer has been used as a specific and sensitive measuring instrument for sulfur dioxide, hydrogen sulfide, and methyl mercaptan [26]. The gas chromatographic system has been readily modified to measure a wider variety of organic sulfur compounds where appropriate, such as in the vicinity of Kraft Paper Mills [27].

Several varieties of electrochemical or electroanalytical techniques have been utilized in sulfur oxide monitors [28]. Research and development continues on these approaches. Electroanalytical techniques are rarely specific. Liquid or solid substrates are often used to confer specificity. One type of electrochemical transducer utilizes a selective membrane along with a galvanic cell having an electrode potential selected to reduce interferences. Work has been done on selection of electrode and electrolyte materials and membranes for development of sulfur dioxide and nitrogen oxide analyzers [29]. This sulfur dioxide analyzer is a compact, relatively low-cost instrument. Its sensitivity at present is adequate for short-term emission measurements but is marginal for continuous measurement of sulfur dioxide. The sensor utilizes 1.0N  $\text{H}_2\text{SO}_4$  electrolyte, a gold sensing electrode, and a lead dioxide counter-electrode with a 1-mil polyethylene membrane. With potentiometric control, interference from nitric oxide is low, but hydrogen sulfide constitutes a significant interference. Response time is less than a minute. This analyzer requires improvement for use in ambient air analysis. Because of its mode of operation, simplicity of construction, compactness, and low cost, it offers possibilities for use in large monitoring networks for measurements averaged over extended time intervals, such as 24-hour periods.

Measurement of nitric oxide in the ambient atmosphere has involved use of analyzers utilizing the colorimetric Griess-Ilosvay reaction for nitrogen dioxide [29], in which the nitric oxide is oxidized to nitrogen dioxide before analysis. A number of efforts have been made to overcome difficulties in developing an oxidizing substrate capable of providing high conversion efficiencies over a range of atmospheric conditions. The stoichiometry of the colorimetric nitrogen dioxide reaction used has been in dispute [30]. In addition, the nitric oxide and nitrogen dioxide colorimetric analyzers have been difficult to keep in proper operation. No electroanalytical or ultraviolet analyzer has yet been provided that is adequate for ambient air monitoring of nitrogen oxides. The need for improved instrumentation is particularly urgent for nitrogen dioxide.



At present the most promising approach involves the use of chemiluminescent emissions from the electronic transition,  $\text{NO}_2^* \rightarrow \text{NO}_2 + h\nu$ , produced in the reaction of nitric oxide with ozone [22]. The emission spectrum extends from 0.6 to 3 micrometers, with maximum intensity near 1.2 micrometers. This method for nitric oxide determination is linear from 0.004 ppm up to 10,000 ppm. Photomultipliers responding from the outset of emission at 0.6 micrometer to their cutoffs at 0.8 to 0.9 micrometer have been evaluated. To obtain a sensitivity down to 0.004 ppm, choice of photomultipliers must be optimized and the operating temperature of the photomultiplier reduced to  $-20^\circ\text{C}$ . Since such sensitivity is not needed in measurement of source emissions, less sensitive photomultipliers operating at room temperature can be used. For a wide range of gases present in polluted air, quenching of the chemiluminescent reaction has not been observed at reduced pressures. No interfering species have been identified. This same reaction has been mentioned earlier as a method for ozone measurement.

The reaction of nitric oxide with atomic oxygen produces excited states that emit weakly, with some line structure occurring between 0.35 and 0.45 micrometer, followed by a more intense unstructured band with a maximum near 0.65 micrometer [32]. Since atomic oxygen reacts with nitrogen dioxide to form nitric oxide on a one-to-one basis, this reaction can be used to measure  $\text{NO} + \text{NO}_2$ . This reaction can be used as the basis of a rapid-response instrument for measuring nitrogen oxides. Use of the emission in the 0.65 micrometer region only provides discrimination against the chemiluminescent emissions produced by the corresponding reactions of atomic oxygen with carbon monoxide and sulfur dioxide [32].

Instruments have been fabricated for monitoring nitric oxide in ambient air by reaction with  $\text{O}_3$ , and  $\text{NO}_x$  by reaction with atomic oxygen [33,34]. Field evaluation of these instruments will follow.

Optical techniques based on absorption of energy by pollutant molecules in the ultraviolet, visible, infrared, or microwave regions have found little application in ambient air monitoring. An exception is the non-dispersive infrared carbon monoxide analyzer. An infrared fluorescent analyzer has been developed for CO that has high sensitivity and specificity [35]. Other pollutants existing at lower concentrations do not absorb sufficient energy to permit utilization of other present commercial types of NDIR atmospheric analyzers. Improvements can be achieved by multiplexing (the simultaneous observation of multiple spectral resolution elements), which can be used to obtain higher signal-to-noise ratios [36]. Multiplexing can be obtained in a variety of optical devices, including

multiple-channel radiometers, interferometers, dispersive matched-filter spectrometers, grating spectrometers, and cross-correlation nondispersive analyzers [36]. More use of this technique has been made in remote-tuned closed-path instrumentation, but multiplexing can be used to advantage in both types of applications.

In recent years, some of these optical techniques have been developed with specific application for ambient air analysis. These approaches involve either differential spectrophotometry or correlation spectroscopy. The technique of correlation spectrometry involves molecular absorption, in which a portion of the ultraviolet or visible spectrum containing vibrational-rotational band spectra is compared with a replica of this spectral region stored within the spectrometer. Point-sampling type correlation spectrometers for  $\text{SO}_2$  and  $\text{NO}_2$  with an optical path length of 2.5 meters have been fabricated and evaluated in Toronto and Los Angeles [37,38].

The second-derivative spectrometer electromechanically processes the transmission *versus* wavelength function to produce a signal that is proportional to the second derivative of this function. This technique results in enhancement of signal-to-noise ratio and improved specificity. A spectrometer of this type has been fabricated to analyze nitric oxide, ozone, sulfur dioxide, and nitrogen dioxide in the 190 to 400 nanometer region [39]. Sensitivities ranging from 0.003 ppm for NO and  $\text{SO}_2$  to 0.02 ppm for  $\text{NO}_2$  are reported for a 1-meter white cell with 20 passes. Both techniques make possible greater specificity by taking advantage of the details of band structure. They also provide somewhat improved sensitivity. However, the electronic and optical systems utilized can be involved and expensive. It is not clear whether these types of optical systems will be competitive in response characteristics, field performance, or cost-size characteristics with the instruments discussed previously for ambient air monitoring.

Another approach is the use of a folded laser beam with white-cell optics. With a laser, a long narrow glass pipe could be used rather than the usual large heavy steel tank [36,40]. To obtain the sensitivities needed for ambient levels of pollutants, at least a 1-kilometer path is needed, requiring a 6-meter base length. Since both an analytical and a reference wavelength are needed, tunable lasers are highly desirable. The tunable semiconductor diode lasers, such as the lead-tin-telluride laser now under evaluation, is attractive because of its small size and extremely high resolution that results from continuous tuning over several tenths of a wavenumber around 10 micrometers [36,41]. Because of its small size, this type of laser plus a detector with excellent sensitivity, such as the

mercury-cadmium-telluride detector in a common liquid nitrogen cooler can be used as the key elements in a closed-path optical instrument [36]. A spin-flip Raman laser providing tunable infrared radiation has been used to measure nitric oxide in the ppm range [42].

All of the instruments or concepts discussed have involved point sampling; that is, the atmospheric sample is pulled into the instrument through a probe or inlet line of some type. Such types of instruments may or may not integrate over time, depending on response characteristics or deliberate provision for integration of the sample or the signal over time. Integration over space is not involved, except as meteorological conditions influence the flow of pollutants past the sampling site. The measurements obtained are not necessarily representative of pollutant levels over extended areas of the city or over entire air quality regions.

A completely different approach to ambient air analysis involves the use of long-path or remote optical measurements. Such optical instruments would provide the ability to integrate over space in two or in three dimensions.

The Research and Monitoring Office of the Environmental Protection Agency has not only a program for development of point-sampling instrumentation, but also a program for development and evaluation of long-path and remote instrumentation. Correlation spectrometers have been evaluated in several cities for long-path measurement of sulfur dioxide and nitrogen dioxide, using near-ground-level paths with two-ended systems utilizing active sources of energy [38]. Path lengths from 100 meters up to 1000 meters were used in these measurements. In the work done in Los Angeles, two path lengths were compared, one a cross-freeway path and the other an off-freeway path. Long-path results were compared to point-sampling results, but the experiment also showed that point sampling could be unrepresentative of the average pollutant concentrations even in a 1000-meter path. These measurements can be limited by aerosol scattering effects or excessive sunlight [38]. An open-path circular-filter-type infrared analyzer, programmed for ozone measurement but capable of measuring other pollutants in the 7 to 14 micrometer range, has been developed [43]. This instrument is designed to measure ozone between 0.01 and 1 ppm at path lengths from 400 to 1600 meters. Another investigation being supported is concerned with the potential of a CO<sub>2</sub> laser system for long-path measurement of ambient air pollutant concentrations [44].

The optical instruments discussed thus far involve either two-ended systems, or use of reflectors over fixed path lengths. Ideally, the open-path system of choice might involve a pulsed signal with ranging capabili-



ty. If such a system could be developed with sufficiently extended range, it could cover much of an urban area in three dimensions. Such a system would probably require a highly developed pulsed laser system with complex data-handling capacity. A LIDAR-type system of this sort has received some evaluation by several groups for estimating total particulate loadings in the atmosphere [45,47]. Similar systems do not presently exist for measurement of gaseous pollutant molecules. The use of laser Raman spectroscopy offers possibilities, but sensitivities appear marginal at present [48]. Considerable augmentation of the scale of the present efforts will be necessary to develop practical systems for routine monitoring activities.

Still another area of interest involves the use of remote optical instruments mounted in aircraft. The instrumentation can be pointed at the earth's surface to measure the albedo of the multiple path of sunlight passing through the atmosphere to the surface and reflected upwards to the aircraft-mounted instrument. Correlation spectrometers for sulfur dioxide and nitrogen dioxide have been evaluated in flights over several cities [38]. This technique also has applicability to monitoring of stack emissions and plumes [38]. However, several problems exist in applying the technique. In a clear atmosphere the equipment tends to operate reasonably well, but scattering by aerosols limits use of the equipment and also renders uncertain the appropriate path length to include in the calculation. In addition, such aircraft measurements are limited by any atmospheric conditions that interfere with flight operations.

If aircraft operation of remote instrumentation is possible, various earth-oriented satellite applications also are possible. Such satellites would not offer the flexibility, accuracy, and general utility of aircraft in taking measurements over urban areas of air quality regions. However, satellites may have eventual utility for measuring long-lived pollutants such as carbon monoxide and submicrometer particulates on a global scale, although adequate measuring techniques are still lacking at the present. NASA is supporting effort particularly for methods that analyze carbon monoxide by utilizing both infrared correlation spectroscopy and nondispersive infrared techniques. The latter approach was selected as applicable after a survey on numerous trace gas detection techniques [49]. Applications of optical techniques in the upper atmosphere of the earth or to other planetary atmospheres will not be considered in the discussion.

The chemiluminescence ozone, and the gas chromatographic carbon monoxide and methane instruments have already been used in low-concentration-level geophysical measurements [50,51]. The flame photomet-



ric sulfur oxide and the chemiluminescence nitrogen oxide monitors may be usable for nonurban or rural measurements, but do not presently have the sensitivity for geophysical measurements. The initial emphasis has been on development of equipment capable of continuously or periodically measuring pollutant concentrations in samples of ambient air collected in a very short time interval. However, for urban measurements as well as nonurban and geophysical studies, integration of concentrations over time periods of 1 to 24 hours is an alternative to the conversion of continuous measurements to averaged values for these periods by data reduction techniques. Work is just being initiated on the possibility of utilizing several of the available types of sensors for integrated measurements.

Monitoring capability is especially important for carbon monoxide, sulfur dioxide, hydrocarbons, ozone, and nitrogen dioxide because air quality standards for these widespread pollutants will be established throughout the United States [52]. In addition, a number of other gases or vapors are of concern. These substances include hydrogen sulfide, organic sulfur compounds, hydrogen fluoride, hydrochloric acid, chlorine, and nitric acid. With the possible exception of hydrogen fluoride (and water-soluble fluorides) the lack of data makes it difficult to estimate the levels or wide-spread prevalence of these substances.

As already discussed, a sensitive and specific gas chromatographic technique is now available for hydrogen sulfide and organic sulfur compounds [26,27]. Hydrogen fluoride is generally measured as fluoride along with other water-soluble fluorides. However, a fully satisfactory monitoring instrument does not appear to be available. The ion-selective fluoride electrode may have the greatest potential because it has been reported to have acceptable selectivity and reproducibility [54]. Colorimetric methods utilized have required considerable processing, including distillation, because of poor specificity. The ion-selective electrode has not yet been incorporated into an operational field instrument.

No methods are available that have satisfactory specificity for hydrochloric acid, chlorine, or nitric acid. Hydrogen chloride is emitted from combustion of chlorine-containing coals and incineration of chlorine-containing materials, particularly plastics, and this acid may also be formed by atmospheric photooxidation of chlorinated solvents. Therefore, hydrochloric acid may be more prevalent than is often assumed.

Nitric acid is a direct emission product from nitric acid manufacturing, but a much more important source may be photochemical reactions. Very little of the nitric oxide emitted is ever accounted for in the form of particulate nitrate. Nitric acid has been shown to be an important product of

photooxidation of nitric oxide in the presence of hydrocarbons in laboratory experiments [55]. The kinetic mechanisms usually postulated for conversion of nitric oxide and nitrogen dioxide to products also favor nitric acid formation. Because of a lack of an acceptable technique for atmospheric analysis, we cannot assess the importance of nitric acid as an atmospheric pollutant. Optical techniques, including open-path instrumentation, should offer possibilities for measurement of nitric acid, hydrochloric acid, chlorine, and hydrogen fluoride.

Thus far, the discussion has greatly emphasized inorganic pollutants. In part, this reflects the development of air quality criteria for only one group of volatile organic substances — nonmethane hydrocarbons [52]. The lack of additional differentiation among volatile organic substances does not result so much from lack of laboratory analytical methods as from lack of routine monitoring techniques.

Gas chromatographs with flame ionization detectors have been utilized in mobile laboratories to analyze atmospheric samples for identification and quantitation of 30 to 60 different aliphatic and aromatic hydrocarbons [56,57]. The chromatographs have been used both for periodic monitoring and for grab sampling. The number of components analyzed has depended on the substrates used and the atmospheric concentration levels. Process-type gas chromatographs for nonmethane hydrocarbons are in development.

Peroxyacetyl nitrate also has been analyzed by gas chromatography with electron-capture detectors [58]. Because peroxyacetyl nitrate is unstable, grab sampling is not satisfactory; however, on a given site monitoring can be conducted. Calibration problems and the lower stability of the electron-capture detector considerably limit the utilization of this technique by monitoring networks. Formaldehyde, acrolein, and aliphatic aldehydes also have been analyzed in atmospheric samples by colorimetric techniques [6]. Results reported have involved manual sampling and analysis. Colorimetric analyzers have been fabricated and used in some field applications [59,60]. Unfortunately, these colorimetric analyzers, and other analyzers based on colorimetric techniques, would present considerable problems in large-scale routine monitoring use because of the need for reagent preparation and use of liquid flow systems, and because of varying response to individual aldehydes.

The monitoring that has been done for specific organics has been done by a limited number of groups interested in improving understanding of atmospheric chemistry or in identifying the contribution from various emission sources to hydrocarbon composition. Air quality standards now being established are not likely to require differentiation among these organic species.

Analysis of nonmethane hydrocarbons has several fundamental limitations: (1) the measurements cannot be utilized to determine the effectiveness of control, over a given period, of any particular source of organics; (2) response characteristics of the flame ionization detector differ for various hydrocarbons; and (3) the flame ionization detector does not respond to formaldehyde and shows reduced response to other aldehydes.

Even a detailed gas chromatographic analysis for hydrocarbons will not make it possible to follow control of even the major sources of hydrocarbons. This difficulty results from a lack of specific hydrocarbons that can be used as unique tracers of contributions from individual emission sources. However, acetylene and ethylene can be used as indicators of control of combustion sources. In downtown high-traffic-density areas, these hydrocarbons also should serve as indicators of the level of hydrocarbons from vehicular exhaust. Ethylene measurements would be of direct interest in some areas because of its plant-damage characteristics.

Some steps must be taken to optimize flame ionization detector response to minimize deficiencies in response to individual hydrocarbons in atmospheric analysis. Calibration with a single saturated hydrocarbon is the accepted procedure. Such a calibration limits the accuracy of comparative measurements of atmospheres in which the hydrocarbon composition varies.

There is a definite need to include aldehydes in the measurement of volatile organics. Aldehydes can constitute a significant fraction of the reactive organic substances present in atmospheric samples. Better methods for monitoring atmospheric aldehydes are required, however. Optical techniques warrant more consideration for their potential in providing convenient means for monitoring aldehydes in the atmosphere.

## B. INSTRUMENTATION FOR MEASURING ATMOSPHERIC PARTICLES

Particulate matter analysis is more complex and in some ways less advanced than analysis of gaseous air pollutants. Certainly, there is a clear lack of instruments for analyzing particles by mass, size, and chemical composition. The great bulk of available results have been obtained by collecting samples on filters in the field and then weighing and analyzing the collected sample in the laboratory. Particles larger than 10 micrometers settle readily, and they are usually associated with settled dust and dirt. Particles in this size range are deposited in the nasopharyngeal region of the respiratory tract and do not tend to penetrate effectively into the pulmonary and tracheobronchial regions [61]. Consequently, these large



particles have not been associated with toxicological action. Particles above 10 micrometers also do not have significant effects on light scattering and on visibility. Sampling for these large particles has been accomplished with dustfall jars, adhesive coatings, cyclonic collectors (for higher-volume samples), long horizontal tunnels (as fractional elutriators), and various impactors [62]. Dustfall jars appear as satisfactory as more complex and expensive techniques although the dustfall jar has poor time resolution. The justification for the use of dustfall jars has decreased, however, since they have become increasingly poor measures of particulate matter pollution as the level of atmospheric particles smaller than 10 micrometers has increased. In view of these circumstances, little justification exists for directing effort toward development of instruments that can measure particles larger than 10 micrometers.

Particles between 0.1 and 10 micrometers probably are those of the greatest concern in air pollution. The particles in this size range contribute most of the particle mass. These particles penetrate into the pulmonary and tracheobronchial regions. The proportion of the particles that are between 0.1 and 1 micrometer is particularly important. Particles in this size range are largely responsible for reduction in visibility, and for haze and turbidity. Studies of physiological response to particulate matter indicate that particles smaller than 1 micrometer can have greater irritant potency than larger particles [43].

Measuring techniques for particles in the 0.1 to 10 micrometer range have included the Volz sun photometer, the integrating nephelometer, various other forward-scattering and right-angle-scattering instruments, cascade impactors, electron microscopes and tape samplers [62]. None of these techniques is adequate for providing a quantitative measurement of mass concentration of 0.1 to 10-micrometers particulates. Several of these techniques have been research tools not primarily intended to give an overall measure of mass concentration; nonetheless, they have given valuable specialized measurements on particular characteristics of particles in this size range.

The tape sampler has received considerable use in the measurement of suspended particles. Visual color of the spots on the tape has been compared with a standard gray scale. More frequently, reflectance or transmittance measurements have been used. Transmittance measurements, however, have been shown to relate poorly to the mass of particles. Reflectance also is not ordinarily related to total suspended particulate matter but to "dark suspended matter," inasmuch as reflectance is also a function of absorbance, and absorbance is a function of "color." Both techniques suffer from a number of complications related to variability in the characteristics of the deposits. Neither technique can be considered



satisfactory for measurement of absolute concentrations of particulates, but tape samplers can be used to obtain relative values. Careful standardization is critical. Changes in the characteristics of the particulates over periods of years at a site because of fuel changes or control efforts may limit the usefulness of these measurements even for obtaining relative values.

The integrating nephelometer has received considerable evaluation in recent years in an attempt to relate its response to mass concentration of suspended particulate matter [64-67]. This instrument was designed to measure meteorological range, which in turn correlates well with visual range. However, a close correspondence between nephelometer response and mass concentration obtains only if the particle-size distribution and other particle characteristics remain constant. Unfortunately, the experimental measurements themselves show this not to be the case [68]. A visual range of 7.5 miles can be associated with mass concentrations ranging all the way from 50 to 200  $\mu\text{g}/\text{m}^3$ . Such a range of mass concentrations represents the entire range of annual geometric mean concentrations of suspended matter in urban areas. Similarly, a visual range of 5 miles can be associated with suspended particulate loadings ranging from 75  $\mu\text{g}/\text{m}^3$  to 300  $\mu\text{g}/\text{m}^3$ . Such results clearly indicate that nephelometer measurements are completely inadequate as a means of determining whether particulate emissions comply or tend to comply with air quality standards for particulates. This instrument certainly is useful, however, in its original purpose, which is the measurement of meteorological or visual range. Since light scattering is associated primarily with particles in the 0.1 to 1 micrometer range, it would seem more useful to attempt to relate nephelometer measurements to mass concentration in the 0.1 to 1 micrometer range than to total suspended particulate matter.

The equipment that has received the greatest use in particulate matter measurement has been the high-volume sampler [62]. This device necessitates the replacement of filters that must be transported to the laboratory for weighing and chemical analysis. Ordinarily, a 24-hour-average sample is obtained. When such integrated total weights of suspended particulate are required, the high-volume sampler is an adequate device. However, this sampler can collect particles well above 10 micrometers and may not be too efficient in collecting particles approaching 0.1 micrometer. In addition, the sampling rate is sensitive to the mass of material collected. It is in the utilization of high-volume samples for subsequent chemical analysis that a multitude of problems occur. The glass fiber filter medium as ordinarily used gives high blank readings for a considerable number of cations and anions. Furthermore, reactions can occur on the filter medium between collected materials. Oxidation or volatilization of

some collected substances can and does occur. Other filter materials can be used, particularly for special analytical applications, but such filter materials usually have limitations associated with their use as well.

It should be apparent that these sampling problems cause greater obstacles to overall analytical accuracy than those caused by limitations in the analytical techniques themselves. Conversely, it seems futile to expend much effort on improving analytical techniques if the greater source of uncertainty results from the sampling technique itself.

The Anderson sampler has received considerable use as a particle-sizing device. Although materials collected on the various stages must be returned to the laboratory for weighing or analysis, this sampler can serve as a useful interim approach for both mass and composition measurements.

A number of types of instruments ought to be developed for the measurement of particulate matter. A continuous or periodic monitoring instrument capable of measuring the mass concentration of aerosols below 10 micrometers is needed. An instrument has been developed that uses an electrostatic precipitator to deposit aerosol particles directly onto a piezoelectric quartz crystal microbalance [72]. The balance is sensitive enough to permit measurements of incremental mass for time periods of less than 1 minute. Collection can be significantly influenced by sorption or desorption of water vapor, but this effect can be reduced by use of a dual-crystal detector. Adhesion of larger particles depends on the composition of the particles and the relative humidity. Addition of a surface active agent to the flow of sample can help minimize this difficulty. Because of these possible limitations, such equipment requires careful field evaluation and comparison with normal particle-sizing collection techniques [69-71].

Equipment capable of periodic monitoring of particle-size distribution by mass concentration also is required. An investigation is underway of a new cascade impactor concept for particle-size fractionation, in which beta-ray attenuation is used to measure material collected on a filter tape.

In view of the difficulties in filter collection and chemical analysis, development of on-site chemical analyzers for various chemical species in particulate matter may be justified. Aside from monitoring needs, improvement of our knowledge of gas aerosol transformations in the ambient atmosphere requires instruments capable of the diurnal monitoring of such species as sulfuric acid, total particulate sulfate, particulate nitrate, and organic aerosol. All of these substances contribute to the loading of particles in the 0.1 to 1 micrometer range. Research studies using particle-sizing samplers also have identified lead, sulfate, nitrate, carbon-containing species and ammonium and chloride ions as con-

stituents in the 0.1 to 1 micrometer range [69-71,73,74]. To properly associate visibility with air pollution control effectiveness, both the total mass concentration within the 0.1 to 1 micrometer range and the contribution of these chemical species to the mass concentration must be known. Of course, the direct association with visibility also involves computing the on-site contributions of individual species based not only on mass and size distribution but also on optical characteristics of the particles.

Despite emphasis on automated field methods, laboratory procedures will continue to be useful in particle-size and morphological analysis of airborne particles [75,76]. For example, optical and electron microscopy can be used for particle sizing. These techniques, along with x-ray diffraction and other measurements of physical properties, have been used to characterize a wide variety of types of particles. An atlas of photomicrographs of various airborne particles is available to aid in microscopic identification [77].

The electron microprobe has been used for identification and for estimation of relative concentrations of a variety of elements including Al, Si, P, S, Cl, K, Ca, V, Fe, Ni, Cu, Zn, Br, La, Ce, and Pb in airborne particles [78,79]. In addition, Millipore filters and beryllium disks have been scanned by area for individual elements. Lead has been found associated with bromine and chlorine, but samples also have been scanned in which the lead is present with sulfate. Particles can be located in which no elements can be identified in the x-ray distribution patterns, probably because they contain carbon and other lower-atomic-weight elements.

The monitoring of a variety of hazardous or potentially hazardous elements, including Be, Hg, As, Cd, V, and Mn may require the development of on-site analyzers. For this purpose, x-ray fluorescence techniques should have potential. Work is underway to estimate better the potential of x-ray fluorescence for analysis of airborne particulate fractions. Atomic absorption already has been shown to have potential for continuous analysis of certain elements in air, such as lead [80]. Such equipment would allow sampling each 24 hours or more often, 7 days a week, instead of the more frequent analysis ordinarily associated with filter collections and laboratory analysis. A monitoring technique for various elements also may be desired for analysis of some types of stationary emissions.

### *1. Elemental Analysis*

Particulate matter samples are usually analyzed for the elements after collection on a filter. Contamination of the filter media with trace quantities of the elements is a common limitation on analysis. The glass fiber



filter most commonly used for collection is not satisfactory for trace analysis for a number of metals because of such contamination. Other substrates, such as polystyrene membrane or Millipore filters, have been used but still are far from completely adequate. For a few elements such as mercury, other collection techniques are used, such as amalgamation or use of gas bubblers.

Methods of analysis utilized included colorimetry for a few elements. Usually physical techniques including optical emission spectroscopy, atomic absorption spectrophotometry, neutron activation analysis, x-ray fluorometry, spark-source mass spectroscopy, and stripping voltammetry have been used for elemental analysis. The status of techniques available through 1966 has been reviewed with emphasis on wet chemical techniques, ring oven methods and atomic absorption spectrophotometry [81].

Optical emission spectroscopy has been used for elemental analysis over the past 15 years in Federal monitoring activities. This spectrographic technique has been applied to analysis of 17 elements—As, Be, Bi, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Sn, Ti, V, and Zn [82]. This method involves considerable analytical skill but it is still only semiquantitative, lacks sensitivity for a number of elements, and is limited by blanks in the filter substrate.

Atomic absorption is a more quantitative and specific technique and is very sensitive for some elements. This technique can be used for As, Be, Na, K, Ca, Mg, Ba, Cd, V, Co, Cu, Zn, Ag, Ni, and Pb [70, 83-85]. The method is limited by the availability of hollow-cathode lamps. A number of these elements have been measured after particle-sizing of airborne particulates [70]. Atmospheric precipitation samples have been analyzed for Pb, Zn, Cu, Fe, Mn, and Ni [86].

The thermal neutron activation analysis of particulate matter in air has received increased use in recent years [87,88]. Scintillation counting using a thallium-doped NaI crystal for samples on cellulose fiber was utilized to determine Al, V, Mn, Na, Cl, and Br. In much more ambitious use of neutron activation analysis, airborne particulate samples were collected on polystyrene filters and counted by means of a lithium-doped Ge detector. The procedure permits determination of up to 33 elements provided that 5 minute irradiations are utilized with 3 and 15 minute cooling periods and a 2 to 5 hour irradiation with a 20 to 30 hour and a 20 to 30 day cooling period [89]. Even with this scheme, most of the low-molecular-weight elements cannot be analyzed. The sensitivities for a number of the elements are marginal or inadequate because of various limitations, including interference of other substances in the sample or a high blank



value. Included in the group of elements analyzed by this method are Mg, S, Cl, Ti, Ni, and Ag. Determination of Li, Be, B, C, N, O, F, Si, and P could not be made. Therefore, only a few elements lighter than K could be analyzed. In addition, Pb and Cd, biologically important elements that occur in airborne particulate matter, are not included in the scheme. A number of the elements that can be measured are not presently of concern biologically nor do they contribute significantly to the mass of particulates. These elements include Ga, La, Sm, Eu, Au, Sc, Ce, Co, and Th. Elements such as Cr, Fe, Ni, Zn, Se, Sb, and Hg are determinable only after a 20 to 30 day decay period. Therefore, although this neutron activation analysis scheme in principle appears very attractive, in practice it can only be applied with one or more other analytical techniques if determination of a range of biologically important elements or a mass balance is desired.

A particularly interesting study made recently of elemental analysis of particulates included use of optical emission spectrography, spark-source mass spectrography, x-ray fluorescence, atomic absorption, CHN analysis, and chlorine analysis by a colorimetric technique [79]. By this means, 77 elements could be determined or estimated in particulates collected in Cincinnati, Denver, St. Louis, Washington, Chicago, and Philadelphia. Bound oxygen could not be included in this analytical scheme. This study is one of the few that permits any evaluation of analytical results from two or more methods on the same samples. The agreement was good between the analyses by atomic absorption and x-ray fluorescence for Ca, Fe, Cu, and Zn, often being within 20 percent or less of each other. The agreement was not as close for K and Pb in some samples. When the analyses for Cu and Zn by optical emission spectrographic technique were compared with those by atomic absorption or x-ray fluorescence, the values obtained by emission spectrography averaged a factor of two to three lower. The Fe analyses by the optical emission spectrographic technique also averaged appreciably lower than those by atomic absorption or x-ray fluorescence.

It is likely that even with additional improvements in these techniques, at least two if not three different analytical techniques will be required to cover the range of elements of interest with adequate sensitivity. It also would seem of considerable importance to conduct concurrently some additional comparisons of each of the several techniques for elemental analyses of the same sets of airborne particulate matter samples. If confidence is to be placed in these analyses being correct, on an absolute basis, to within 20 to 30 percent, the reliability of each of the applicable methods to each element of interest must be ascertained.

## 2. *Anion Analysis*

Analyses for sulfate and nitrate are routinely made on bulk particulate matter samples collected on the high-volume sampler at urban and nonurban sites [82,95,96]. Sulfate is determined by the methylthymol blue method by means of an AutoAnalyzer [96]. Nitrate is assayed colorimetrically following reduction to nitrite by alkaline hydrazine [96]. In investigations on the particle-size distribution of sulfate, several analytical procedures have been used. In one of these investigations, high-temperature reduction of sulfate to hydrogen sulfide was utilized, followed by an iodometric, microcoulometric titration for the hydrogen sulfide [74]. In other work [69,97] a modified turbidimetric procedure was used to analyze the size-fractionated material from an Anderson sampler [98]. Nitrate was also determined colorimetrically after reduction following particle sizing [71]. Phosphate and chloride also have been analyzed colorimetrically after particle sizing [71].

## 3. *Organic Particulates*

A considerable amount of analytical effort has gone into the measurement of various types of organic substances in particulate matter. Polynuclear aromatic hydrocarbons, particularly benzo (a) pyrene, have received the most attention. Certain of the procedures involving column chromatography followed by spectrophotometric analysis have interference problems associated with them. Paper chromatography, but more usually thin layer chromatography, have received considerable attention as separation techniques. Spectrofluorometric analysis and quenchofluorometry have been applied to analysis of polynuclear aromatic hydrocarbons, azo heterocyclics, and polynuclear ring carbonyls after use of column and thin-layer chromatography. Gas chromatography has been applied to *n*-alkanes in airborne particulates as well as to polynuclear aromatic hydrocarbons. However, adequate separation of benzo (a) pyrene from benzo (e) pyrene and perylene has been a problem in applying gas chromatographic procedures to the analysis of airborne particulate matter. The details of these developments have been reviewed up to 1968 in several publications [3,4,90,91]. The status of work on pesticides, nonvolatile fatty acids and phenols is also considered in one of these reviews [91].

More recently, a simple and rapid procedure has been developed for the determination of benzo (a) pyrene, benz (c) acridines and 7H-benz [de]

anthracen-7-one [92]. Benzene extracts were separated by one-dimensional thin-layer chromatography followed by analysis of benzo (a) pyrene by spectrophotometry or spectrophotofluorometry and the benz (c) acridines and 7H-benz [de] anthracen-7-one, in trifluoroacetic acid, by spectrofluorometry. The procedure was applied to 6-month composites from 52 cities. Most analyses for polycyclic organic materials are applied to 24-hour integrated samples or composites representing even longer integration times. A sensitive procedure, requiring less than 20 minutes, that involves thin-layer chromatography and direct fluorometric measurement was applied to 3-hour sequential air samples for analysis of 7H-benz [de] anthracen-7-one and phenalen-1-one [93].

Little recent work has been done on pesticides in air. Samples collected at nine urban and rural sites were analyzed for 19 pesticides and their metabolites [94]. Chlorinated pesticides were determined on two different gas chromatographic columns utilizing electron-capture detectors, while organophosphate pesticides were determined on two different gas chromatographic columns utilizing flame photometric detectors. Only p,p'-DDT and o,p'-DDT were found at all locations.

### C. STATIONARY SOURCE EMISSION MEASUREMENTS

Sources of pollutants among stationary sources vary from thermal power plants and incinerators to cement plants and petroleum refineries. Pollutants associated with such sources include particulate matter, sulfur oxides, nitrogen oxides, fluorides, carbon monoxide, and organics.

Emissions of pollutants from stationary sources may involve flue gases or other vented gases. For some types of extended stationary sources analytical measurements would have to be made by a network of sampling sites or by long-path techniques. Such problems have not received much attention in practice.

For flue gases or vented gases in stacks, techniques of measurement do exist. In such situations it is important not only to measure the pollutants, but also the gas flow velocity, excess air, moisture, *etc.* The sampling technique is of great importance and it must be suitable for the specific application. If sampling problems are handled adequately, the analytical measurement requirements can be simplified.

The analytical measurements of pollutants can be accomplished by at least four approaches: (1) intermittent sampling on site with manual analysis in the laboratory, (2) continual instrumental analysis of pollutants collected through probes, (3) in-stack instrumentation, (4) remote instrumental techniques. The second group of instrumental methods would include instruments capable of operating at emission concentration levels and the use of a proper sample dilution system permitting use of ambient air type instrumentation. The in-stack instruments presumably integrate so that probing the stack configuration is not necessary.

Remote instrumentation cannot be utilized continually, but this class of instrument can be used for rapid surveys and checking compliance. The problem of positioning instruments or probes in stacks is eliminated. Problems associated with the correct relationship between in-stack particle concentration and particle size distribution to that emitted out of the stack into dilution air also are avoided. However, the practical development and utilization of remote optical instruments with the appropriate characteristics is difficult, scientifically and technologically.

### *1. Manual Procedures*

Methods in common use for measurement of pollutants from stationary sources of emissions have been manual rather than instrumental. Filters, impingers, evacuated flasks and condensation procedures are the collection techniques commonly used. To express pollutant mass loadings and relate results to process variables, additional measurements are required. These measurements include stack gas velocity with a pitot tube, moisture in-stack gases determined gravimetrically on condensate, excess air by Orsat analysis and carbon dioxide by the nondispersive infrared spectrometric method [99-101].

Sulfur dioxide usually is determined by collection of the flue gas in impingers containing hydrogen peroxide [102-105]. The sulfur dioxide is oxidized to sulfate and analyzed after reaction with barium chloroanilate colorimetrically at 530 nm in terms of the chloroanilate ion. A second procedure often used involves determination of the sulfate by the barium perchlorate thorium titration method [102,103]. The sulfur trioxide or sulfuric acid mist, after separation by filter collection, is determined by the same procedures.

Cations such as  $\text{Al}^{+3}$ ,  $\text{Cu}^{+2}$ ,  $\text{Fe}^{+3}$ ,  $\text{Pb}^{+2}$ , and  $\text{Zn}^{+2}$  produce negative interference in the colorimetric procedure by precipitating chloroanilate ion from solution. These interferences can be minimized by filtering the flue



gas with glass wool at the probe and pretreating the solution with a cation resin. Anions causing interference are not likely to be present in flue gases. The titration with barium perchlorate is interfered with by cations such as  $K^+$ ,  $Na^+$ ,  $NH_4^+$  by reducing the volume of titrant needed. Cationic interferences are minimized by use of a particulate filter in the probe and by percolation of the collected solutions through a cation exchange column. Anions which can co-exist in the collected solutions such as nitrate, chloride and fluoride can interfere also.

These sulfur dioxide analytical procedures have practical ranges from 10 to 3000 ppm by volume. The sensitivities are 10 ppm for 25 to 30 liter samples. The precisions are about  $\pm 3$  percent at 1500 ppm. For sulfuric acid aerosol the range is 10 to 300 ppm with a precision at 10 ppm of  $\pm 5$  percent.

Nitrogen oxides often are collected from flue gases in an evacuated flask containing dilute sulfuric acid-hydrogen peroxide absorbing solution. The nitric oxide or nitrogen dioxide are oxidized to nitric acid which is measured colorimetrically at 420 nm as nitrophenol disulfonic acid [106]. This technique has a range of from 15 to 1500 ppm by volume with a sensitivity of 1.5 ppm. Halogens interfere in this procedure.

In common practice, particulate matter is measured gravimetrically after removal of uncombined water [107-109]. The particulate is removed from the flowing gas stream under isokinetic conditions by filtration and condensation. Impinger collection is used as part of this procedure with extraction of organic particulate matter from the impinger solution with chloroform and ethyl ether. Acetone washing of the probe and filter holder also is included in the procedure. The total particulate weight of aqueous and organic sample components is obtained by totaling the weights of components. Total sample volume or stack velocity and other parameters are utilized in calculating particulate mass in stack gas.

## *2. Continuous Instruments for Sampling Through Probes*

A number of instruments based on optical techniques have potential for use at stack gas pollutant concentration levels. Nondispersive infrared analyzers or ultraviolet analyzers have been designed for sulfur dioxide, nitrogen dioxide and carbon monoxide. Optical techniques have received attention for visibility of stack gases in plumes. Portable electrochemical transducers recently developed have potential for use in measurement of sulfur dioxide and nitrogen oxides in stack gases [29]. The instruments

utilizing the chemiluminescent reaction of ozone with nitric oxide could be utilized for measurement of nitric oxide in stacks [22,31-33].

Gas chromatographic instruments have received considerable evaluation for sulfur dioxide, hydrogen sulfide and organic sulfur gases in Kraft mill effluents [26,27,110-112]. Gas chromatographic analyzers also could be utilized to measure carbon monoxide and sulfur dioxide in various stack gases.

A source sampling technique for particulate and gaseous fluorides involves use of a heated glass probe to convert hydrogen fluoride to silicon tetrafluoride [113]. This type of sampling procedure has been used with a fluoride-selective ion electrode to analyze water-soluble fluorides in stack gases.

An attractive sampling approach would be to convert the stack gases by dilution and cooling to conditions approaching those in ambient air analysis. The same instruments could be used as for air quality measurements. This approach would considerably reduce the number of types of instruments in use, calibration requirements and maintenance problems. Studies are needed on the appropriate sampling interfaces to provide dilution and cooling without changing pollutant composition. Work is in progress on the evaluation of sulfur oxide and nitrogen oxide analyzers for stack gases.

### *3. In-Stack Instrumentation*

Instruments capable of operating within the stack itself usually have been based on optical principles. Such equipment must be built to withstand dust, heat, corrosion and vibration. Thermal gradients can cause considerable problems in optical alignments. Calibration of such instruments requires spectra under experimental conditions closely simulating stack conditions.

### *4. Remote Stack Instrumentation*

Remote sensing techniques offer several advantages over the traditional methods of sampling through a probe introduced into a source of emissions. These advantages are as follows: (1) more representative sampling by virtue of spectral integration across the diameter of a stack plume, (2) no need for interfacing between stack and analyzer with probes

and sample conditions (3) capability of measuring across an extended source such as an oil refinery.

Electrooptical techniques can be utilized remotely to characterize particulate and gaseous emissions. The use of LIDAR systems for determining the opacity of plumes from power plants has received considerable attention [114-117]. A study of the optical properties of such plumes concluded that the optical transmission of the plume best characterized the aerosol loading in emissions. A LIDAR system has been fabricated as a research tool for field studies. More practical field equipment will probably utilize the signal backscattered from the plume aerosol rather than the signal backscattered from the ambient air beyond the plume. Such equipment would use a low powered laser. Mass loading measurements of aerosols in plumes also can be made by LIDAR but interpretation of the signal would require measurements on other characteristics including particle size distribution.

The measurement of gases such as sulfur dioxide can be approached by use of ultraviolet transmission, infrared emission or Raman scattering.

Field evaluations have been made utilizing correlation spectroscopy in the ultraviolet [38] and will be made with high resolution infrared emission spectroscopy. Nitric oxide presents a particularly difficult problem because of the overlap of the nitric oxide bands in the  $5\mu\text{m}$  region by water vapor bands.

Raman scattering provides a means of measuring nitric oxide without serious interference by other stack gas constituents. In a preliminary feasibility study, insufficient signal to noise ratio was available [118]. Improvements in signal to noise ratio can be achieved by use of resonance Raman scatter and by fluorescent scatter techniques.

Detailed analysis of particulate matter in stationary source emissions for elements or compounds presents many of the same problems as those already discussed for atmospheric analysis. Elemental analysis has been limited until the recent increase in concern about trace metals. However, available techniques already discussed for atmospheric particulate samples appear adequate for most stationary source applications. A reasonably extensive sampling and analysis program was conducted some years ago to analyze up to 10 polynuclear aromatic hydrocarbons in samples collected from a variety of combustion processes and industrial processes [119]. Analyses were made by ultraviolet-visible spectrophotometry on the benzene soluble fraction of the samples following separations by column chromatography.

### D. MOBILE SOURCE EMISSION MEASUREMENTS

Research measurements of the detailed composition of emissions of motor vehicles have been made for at least 15 years although emission standards for hydrocarbons in blowby gases were not promulgated until 1963 in California and for exhaust hydrocarbons and carbon monoxide in 1966 in California and in 1968 for the United States. Subsequently, nitrogen oxide standards have been established. Much more restrictive standards for hydrocarbons, carbon monoxide and nitrogen oxides will apply to 1975 and 1976 model year vehicles. Evaporative loss controls also have been established. The limiting factor on implementing standards has not been analytical methods, but control technology applicable to mass production vehicles. The original techniques used for hydrocarbons, carbon monoxide and nitrogen oxides involved the use of nondispersive infrared analyzers. The infrared analyzers for nitrogen oxides have suffered from water vapor interference as well as limitation in sensitivity.

Many techniques have been applied for detailed analysis of hydrocarbon emissions. These have included mass spectroscopy, dispersive infrared analysis, and by coulometric and colorimetric methods [6]. Gas chromatography was applied to automobile emissions shortly after its first use in the United States. However, thermal conductivity detectors were limited in sensitivity and suffered from water vapor and carbon dioxide interference. Chemical pretreatment to remove interferences and concentration techniques greatly complicated the practical application of gas chromatography to automobile exhaust emissions in the late 1950's. In the early 1960's, the application of flame ionization detectors eliminated these earlier limitations and accelerated the use of gas chromatography to measure the detailed hydrocarbon composition of automotive emissions [120,121]. With gas chromatographic capability well established other analytical methods have not been utilized in recent years. The flame ionization analyzer has replaced the nondispersive infrared analyzer as the motor vehicle certification technique for hydrocarbons. The non-dispersive infrared technique was of somewhat limited sensitivity for the more restrictive standards and its response depended on composition in an undesirable manner.

Subtractive columns have been used for analysis of paraffinic, olefinic, and aromatic hydrocarbons in vehicle emissions using a flame ionization analyzer [122]. This technique provides a more rapid approach to class analysis of emission than does gas chromatography.

The use of open-tubular columns combined with solid absorbant or packed columns combined with temperature programming makes it possi-



ble to make about any analysis desired for individual hydrocarbons in emissions. Considerable work has been done applying gas chromatographic techniques to organic oxygenates in exhaust. Recently a chemical ionization mass spectrometer has been demonstrated to be capable of measuring a number of aldehydes and ketones in exhaust. Work also has been done on an electrochemical approach to aldehyde analysis, but this technique is not far advanced.

Nitrogen oxide analysis primarily for nitric oxide has been done by several other analytical techniques in addition to the nondispersive infrared analyzer. These other approaches included use of an oxidizing step prior to colorimetric analysis or use of an ultraviolet analyzer. A mass spectrometric analyzer for nitric oxide also was developed. More recently the need to measure the lower concentrations of nitric oxide required by future standards with use of constant total volume samples has stimulated development of more sensitive analyzers. Electrochemical and particularly chemiluminescent types of analyzers have received recent evaluation.

The chemiluminescent analyzers involving gas titration of nitric oxide with ozone are highly specific, very sensitive and have very rapid response times. A thermal decomposition stage has been used to decompose any nitrogen dioxide to nitric oxide. A closely related technique involves gas titration with atomic oxygen which results in equal responses for nitric oxide and nitrogen dioxide.

The nondispersive infrared analyzer for carbon monoxide has had adequate sensitivity, specificity and speed of response for vehicle emission applications. Therefore, there has not been much incentive to develop other analyzers. Gas chromatographic analysis for carbon monoxide and the recent fluorescent infrared techniques provide more sensitivity if needed.

All of the instruments or methods discussed previously have been developed either for research, certification or surveillance needs. Inspection of motor vehicles or production line testing requires simple and inexpensive instruments. Some of the instruments discussed are useable for these purposes although they are more expensive than desired. Catalytic techniques have been shown to have potential. A simple, rapid response optical instrument of moderate cost also has much appeal.

Considerable effort has gone into analysis of oxygenated hydrocarbons, particularly aldehydes and ketones in internal combustion engine exhaust [6,120,123,124]. Gas chromatographic and colorimetric methods have been emphasized. Phenols also have received attention as products in au-

tomobile exhaust [125,126] by chromatography and colorimetric techniques.

Polynuclear aromatic substances, particularly the hydrocarbons, have been the subject of several measurement projects on automobile exhaust [91]. The work cited on polynuclear aromatic hydrocarbons for stationary sources also included analyses for passenger cars and trucks [119]. Recently, additional work has been done associating polynuclear aromatic substances and phenols with fuels and fuel additives and engine variables [127]. The presence of azo heterocyclic hydrocarbons in automobile exhaust has been demonstrated [128].

Earlier work on particulate matter in automobile exhaust was concerned with amounts of lead-containing particles [129]. During the same period lead was determined polarographically on each of a series of particle sized fractions from auto exhaust [30] from the Anderson sampler, the Goetz spectrometer and other devices. Total and benzene-soluble particulate matter have been measured in two investigations [131,132] on exhaust from a number of passenger vehicles and trucks. Auto exhaust particulate matter after size fractionation in an Anderson sampler has been analyzed for lead, nitrate, sulfate and chloride by atomic absorption and by nephelometric or colorimetric procedures for the anions [133]. The ratios of water-soluble to water-insoluble lead also were compared. An Anderson sampler as well as a constant volume sampler were used to obtain total particulate matter and size-fractionation with analysis for lead, iron and zinc by atomic absorption and bromine by neutron activation [134]. Both leaded and unleaded fuels were utilized. A tunnel type sampling system capable of sampling auto exhaust for particulate matter under realistic operating conditions has been developed [135]. This system was utilized with the Anderson sampler and the Monsanto impactor to do light particle size distributions as a function of vehicle operation conditions and driving history [135]. A similar sampling system was used in a more comprehensive study of composition of particulate and gases with fuels with various tetraethyl lead contents [136]. Total weight, size distribution and metal and nonmetal analysis on exhaust particulate matter and on fuel and engine oil by optical emission spectroscopy, and neutron activation, as well as particle characterization by electron and light microscopy, and organics by mass spectrometric and ultraviolet fluorescent analysis were all included in the measuring techniques applied in a recent study of auto exhaust [136].

Diesel exhaust emissions usually are considered of concern because of smoke and odor problems in the vicinity of individual vehicles. Levels of carbon monoxide are very low from diesels [137]. Hydrocarbon concen-

tration levels can vary widely [137]. Because of the higher molecular weight of diesel fuels, the combined emissions of fuel components, low molecular weight cracked hydrocarbons as well as partially oxygenated organic products presents a substantial analytical problem [120]. A portion of these organic components are responsible for the odors associated with diesel exhaust emissions [138-140]. Nitrogen oxides also can vary considerably in diesel emissions overlapping the concentration levels produced by vehicles with spark ignition internal combustion engines [137]. Polynuclear aromatic hydrocarbons also have been measured at substantial concentration levels in diesel exhaust emissions [141].

Measurement of the concentration levels of a number of components such as carbon monoxide, nitrogen oxides, low molecular weight hydrocarbons and polynuclear aromatic hydrocarbons does not present substantially different analytical requirements from those well established for spark ignition internal combustion engines. Sampling and analysis of fuel hydrocarbons and their partial oxygenated products still present opportunities for analytical activity [120]. Although substantial progress has been made in recent years on identification of the odorous components of diesel exhaust, much more analytical work is needed [140].

Considerable use was made in earlier work by Scott Research Laboratories of dispersive infrared measurements for carbon monoxide, carbon dioxide, nitrogen oxides and some hydrocarbons [138]. Nondispersive infrared instruments were used in Bureau of Mines investigations for carbon monoxide and nitric oxide while nitrogen dioxide was measured with a nondispersive ultraviolet analyzer [137]. Colorimetric methods have been used for nitrogen oxides, formaldehyde, acrolein and total aliphatic aldehydes [138]. Gas chromatography has been used for analysis of hydrocarbons in combination with column chromatography with identification by mass spectrometry for odor components [140]. Considerable progress on odor has been made by this combination of techniques when applied to the oily-kerosene odor fraction and similar techniques are being applied to the smoky-burnt fraction. Column chromatography combined with fluorescence spectroscopy has been used to identify and measure a number of polynuclear aromatic hydrocarbons in diesel exhaust emissions [141].

The use of high molecular weight fuels presents a special problem with respect to the actual form of the higher molecular weight products upon emission. These materials may be present in the atmosphere as vapors, as finely divided organic aerosol, or as large droplets that settle to the ground rapidly. Additional investigation is needed to properly define this situation under realistic operating conditions.

While smoke and odor have been the main concern from the standpoint of possible regulations, other emissions may well have to be included in emission regulations in the future. If emissions from the passenger vehicle equipped with spark ignition internal combustion engines are very effectively controlled by the middle 1970's, the residual contributions from other propulsions systems such as the diesel engine may well become of greater concern.

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## PANEL DISCUSSION

### Analytical Problems in Air Pollution Control



#### CHAIRMAN

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#### PANEL

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Philip W. West, Professor of Chemistry, Louisiana State University, Baton Rouge, Louisiana

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A. P. Altshuller, Director Division of Chemistry and Physics, Environmental Protection Agency (Speaker)

**McCrone** — The optical microscope may be considered a specific sensor. It provides the means to recognize a specific pollutant or to get some characterization data, using the eyes for identification purposes.

Three kinds of problems may be defined. The “needle in the haystack” analysis involves looking at a large number of particles — perhaps millions of them — to find any having a particular composition. They might be asbestos particles, quartz particles, or lead particles. The problem, for example, may be to look for lead particles in an urban atmosphere.

Sometimes, the same type of sample may be given and the analyst is asked to search for “anything that could cause trouble.” Here, trouble has to be defined in terms of who is paying the bill. It might be concerned with hard particles that would harm a polishing operation or it might be concerned with public health. Often, it involves several things. In any event, the analyst would look at the whole sample for any compositions that would cause the specified kind of trouble.

Finally, there is the problem of looking at a settled or suspended dust sample to identify “everything in it.” This is the situation when someone wants to know what dust is settling in his area, or what are the particles he is breathing. Most of the components would be innocuous, but all need to be identified.

Most particles can be identified by optical microscopy if one has seen them before (with a name-tag). For example, asbestos particles, unless very small, have a characteristic morphology and optical properties so there is no difficulty in identifying them. Extensive experience increases the number of identifiable substances. Prepared slides of known materials and reference sources such as the “Particle Atlas”<sup>1</sup> are invaluable aids. Particles as small as a few  $\mu\text{m}$  can be identified by an experienced microscopist.

Electron microscopy offers additional opportunities. Scanning electron microscopy (SEM) is a relatively simple technique which is very popular because the sample requires very little preparation. Many modern SEM’s have solid-state detectors so that they can make x-ray fluorescence measurements, and become in a sense, then, electron micro-probes. With such an instrument one can make a morphological selection of a particle, measure the x-ray wave lengths generated by the electron beam striking it, and thus determine its elemental composition.

The transmission electron microscope has better resolving power than the scanning electron microscope and can identify even smaller particles.

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<sup>1</sup> McCrone, W. C., Draftz, R. G., and Delly, J. D., “Particle Atlas,” Ann Arbor Science Publishers, Ann Arbor, Michigan (1967).

However, the techniques involved are more difficult. Neither electron microscope, of course, is a field instrument.

X-ray diffraction is a means of determining the crystal form of a compound. Even though a particle may be identified as  $\text{TiO}_2$ , it could still be rutile, anatase, or brookite. Optical crystallography or x-ray diffraction would answer this question but the latter is probably easier for most analysts. Fairly simple modification of x-ray cameras permits investigation of particles down to  $10^{-10}$  grams (2-5  $\mu\text{m}$  in diameter). Measurements at the nanogram level are routine. Particles of 2  $\mu\text{m}$  or larger diameter, mounted on glass fibers and centered in the x-ray beam provide measurable x-ray diffraction patterns.

The electron microprobe (EMA) can make an essentially complete analysis of all particles in a sample. You could also use it for "needle in a haystack" analysis. EMA requires about a minute-per-particle to obtain a quantitative analysis. The instrument may be programmed for automatic scanning to look for a combination of elements such as found in a crystal of specific interest. In scanning the sample, it will recognize and analyze particles containing the desired combination of elements.

Particles may be mounted on a beryllium block, to which most microprobes are insensitive. The entire sample or array of particles may be photographed with selected x rays of one of the constituent elements. Thus a picture may be taken first with the x rays of lead to show the lead-containing particles and then with back-scattered electrons to show all particles in the field.

The ion probe (IMA) may be the ultimate analytical instrument. It comes as close to ion-by-ion or atom-by-atom analysis as anything we have and will detect as few as 100-1000 atoms of any element. Accordingly, it is capable of analyzing particles down to a few nanometers in diameter or about  $10^{-17}$ - $10^{-18}\text{g}$ . The IMA determines elemental composition by measuring the relative numbers of isotopes of all elements in the periodic table.

In all of the above methods, a reliable means of collecting samples is obviously required. The sample must be representative to be worth analyzing.

**Mueller** — The technology of particle analysis is the one most in need of further development. No instrumentation presently exists for continuous analysis so that laboratory determination of field-collected samples is currently practiced. The collection of representative samples offers many problems and the collection of liquid particles is a major problem.

The sampling procedure may discriminate on the basis of particle size. For instance, large particles may have too much momentum to change direction to enter a sampling tube and hence may be excluded from a sample. A sampler, consisting of rotating rods or plates, normal to an air stream, is capable of collecting large particles. However, for these collectors the nature of the surface that would optimize efficient collection in the requirements of subsequent chemical analysis needs further investigation. The cascade impactor deliberately changes the air speed and direction to achieve sample size fractionation, based on changes of momentum.

Studies have been made in California to determine elemental compositions of particles as related to the size distribution. Samples have been collected with the cascade impactor and analyzed by fluorescent measurements of x rays generated by alpha particles from a cyclotron. This work was done in cooperation with Dr. Tom Cahill of the University of California at Davis. A sampling time resolution of two hours coupled with sizing into five classes was possible.

This work was concerned with identifying the source of particles. Is a particular haze man-made, natural, or is it a combination? If the latter, how much of each is involved?

Chemical analysis for sodium and chlorine has shown that certain size fractions have a marine origin. Lead and bromine determinations have shown that most of the lead in the submicrometer size arises from automobile exhaust. Vanadium determinations identify particle sizes arising from power plant emissions.

Measurements such as bromine-lead ratios provide insight to atmospheric reactions. It has been found that this ratio is higher at night than during the day, thus indicating a photochemically initiated mechanism for bromine disappearance, involving a change from the particle to the gas phase.

Attempts have been made to investigate compounds occurring in the atmosphere. Unfortunately, wet chemical techniques are too insensitive to determine ammonium, nitrates, and sulfate with sufficient reliability on the small samples available of the fractionation the particles collected in 2 to 4 hours at 50 liters per minute in five size classes. However, photoelectron spectroscopy is now arising as a valuable tool for such determinations. Analyses for lead, nitrogen, and sulfur have been made by this technique in cooperation with Dr. T. Novakov of the Shell Development Company. Normalization of sulfur and nitrogen with respect to lead, and correlation of such data with particle size, provides insights to their origins.



This study also led to the demonstration that four kinds of nitrogen compounds exist in urban particulate matter—amino, ammonium, nitrate, and pyridinium. Nitrate containing particles were found to peak late in the day, indicating a non-automotive source. The other three kinds of nitrogen were found to correlate with automotive traffic patterns. This information, together with the knowledge that ammonium nitrogen is found in exhaust gases and that amino and pyridinium fragments can arise from gasoline additives, implicates automotive emissions as the source of these particles.

**Urone** — There are two areas where the analytical community can help tremendously. One of course is the research area, not only in responding to the everlasting demand for better, quicker, and more specific methods, but also for broadening the horizon in environmental areas by looking for new compounds, new mechanisms, and investigating cause-and-effect relationships between environmental pollution and the receivers, be they people, animals, plants, or materials.

The other important area is in the development of man power. There is a critical need in many areas—local agencies, universities, junior colleges, high schools, *etc.*,—for people with a reasonable knowledge about air pollution. The lack of knowledge in these areas is amazing. The analytical chemist should not only help instruct on methodology but emphasis should be given to the importance of careful analysis. As more people enter the field, the validity and meaning of their measurements becomes increasingly important.

New analytical methods are rapidly emerging. Measurements of pollutants are being made and interpreted by an increasing number of persons, other than analytical chemists. Unfortunately, many of these have vague or incomplete knowledge of the basic analytical principles involved. Accordingly, interpretations made over a period of time may be incompatible. This was illustrated vividly in a recent lecture by Professor Haagen-Smit. He stated that the carbon monoxide concentration in 1967 at seven Los Angeles stations was 11.0 ppm. In 1969, the carbon monoxide level for the same stations was 5.3 ppm. Quite a reduction! This is not true. Los Angeles is getting worse. The explanation is based on the improper use of the non-dispersive infrared method for the measurement. The 1969 measurements had not been properly corrected for interferences by a variable amount of water vapor.

There is a very great need at present for analysts who can identify and measure the organic substances which are produced as primary or secondary products of photochemical and thermal reactions. The processes of

haze formation, the causes of air pollution episodes, such as those of Donora or London, are not well understood. Laboratory experiments tend to repeat the primary photochemical products but do not adequately identify and measure those substances which form aerosols or respiratory irritants in real atmospheres.

**West** — The analytical chemist has been very remiss in letting other people take over his work. I refuse to admit that anyone who wants to call himself an analyst is necessarily qualified to put out numbers that truly represent a situation. Air pollution analysis is tricky stuff and reams of data taken by a black box are not necessarily meaningful just because some pollutant meter is used and masses of data fall out of the box.

An example of a pitfall in pollution analysis is the sampling problem. The high-volume sampler, while adequate for the determination of total mass of airborne material, is not especially well suited for the chemical analysis of particulates. The values for volatile materials such as selenium dioxide are completely erroneous. Also, the filter media themselves are often too contaminated to provide meaningful measurement because of a high or variable blank.

The determination of certain materials in particulate matter is of special interest. Arsenic, chromium, nickel, and asbestos are known to be carcinogenic. Elements which have been indicated as possible carcinogens include beryllium, cadmium, cobalt, lead, and selenium. Manganese is not believed to be carcinogenic but is associated with Parkinson's disease. Even helpful elements such as zinc and copper need to be determined. A sequential tape sampler is excellent for collecting these materials and can provide the 20-30 micrograms needed by modern analytical methods.

The ring oven provides a simple field method for identification and determination of the approximate levels of most of the above-mentioned elements. Essentially it is a solvent extraction separation technique in which the sample is dissolved at the center of a filter paper and carried by a solvent front to the circumference of a heated circle where it collects as a thin but concentrated ring. Sectors of the filter may be cut out and treated with suitable reagents to provide colored ring sections that are compared with standards for their quantitative estimation. Detailed procedures using this technique have been developed in the LSU laboratories for determination of submicrogram quantities of most significant metals, including cadmium, cobalt, beryllium, chromium, nickel, zinc, and lead. Comparisons with other methods, especially atomic absorption spectroscopy, have been very favorable.

**General Discussion** — The analytical requirements of particulate analysis were discussed. Too little effort has been applied to the use of several techniques on the same sample to estimate the accuracy of methods. The lack of suitable Standard Reference Materials makes it impossible to compare results obtained by various investigators.

The requirements of accuracy in air pollution are not severe, at present. The consensus is that errors should be no greater than  $\pm 20$  percent at tolerance levels and results accurate to within a factor of two should be obtained at subliminal levels. The ultimate accuracy may well be related to the sampling problem.

The subject of pollution indices involving a combination of the levels of total particulates and specific pollutants was discussed. The interpretation of such indices is uncertain not only because of the paucity of data but the known fact that what is present is more important than the total amount of suspended material in the atmosphere. Agencies concerned with the state-of-the-environment should be encouraged to support efforts to collect the detailed data needed for more definitive studies of the effects of air pollution. Support of research to develop instrumentation and methodology for the continuous monitoring of specific elements and specific constituents should also be endorsed.







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## CHAPTER 6

# ANALYTICAL PROBLEMS IN WATER POLLUTION CONTROL

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The analytical chemistry of natural and waste waters requires subtle correlation between theory and experience, an insight into the nature and mode of action of interferences, and the ability to interpret analytical results in correlation with pertinent field observations. Because of the complexity of the system under investigation, water pollution characterization is one of the most challenging tasks to confront the analytical chemist.

Design of measurement systems begins with defining (a) WHY the analysis is needed, (b) WHAT are the parameters to look for, and (c) HOW to perform the analysis. Water pollution measurement programs usually incorporate a multitude of physical, chemical and biochemical procedures. The characterization of physicochemical and biochemical transformations in aquatic environments takes into account interactions between atmosphere, hydrosphere and lithosphere and their influence on the aquatic habitat. Trace characterization of persistent organic residues or heavy metals is based on defining their distribution dynamics in the aquatic system, *e.g.*, storage and release by bottom sediments and accumulation and tolerance by aquatic biota.

Recent trends in water pollution measurement rely heavily on advanced instrumental methods and automated analytical techniques. *In situ* sensor systems and remote noncontact optical measurement have been applied for monitoring water quality in rivers, lakes and waste effluents. The effects of environmental factors and interferences on the reliability of the measurement system have been under intensive research.

As America's water pollution problems become more complex, more complex measurement techniques are needed to cope with them. Above all, the effectiveness of pollution control programs are limited by their measurement reliability.

Keywords: Aquatic environment; environmental analysis; trace analysis; water analysis; water pollution; water pollution control.

## I. Introduction

It seems clear to thoughtful individuals that efforts to protect the quality of our environment are highly dependent on our ability for measurement of pollution. **Subjective** responses to environmental quality deterioration in terms of perceived awareness (or annoyance) to such things as taste, odor or noise are ultimately of great significance. Nevertheless, little headway can be made in setting standards, devising quality indices and guarding against pollution of natural resources unless there is a basis in **objective** physical measurement. This implies the ability to measure reliably and quantitatively pertinent water quality parameters, establish baseline values, and monitor changes in these parameters both in location and time.

Present water pollution control activities are limited to a great extent by a lack of adequate measurement capabilities. This is not because the characterization of water pollution is a unique impossible task, but rather, it was only recently when this field gained its present popularity and attracted the attention of analytical chemists. Unfortunately, this situation seems not to be improving fast enough to keep up with the rapidly increasing demands of water pollution control technology. This slow progress, or the lack of it, is to a certain extent the result of the failure of administrators and managers of water pollution control programs to recognize the need for improving present measurement capabilities. As a result, the majority of research and development appropriations in government and industry include insignificant proportions, or a complete absence of allocations, for the development of advanced pollution measurement techniques. Ironically, large amounts of funds are being appropriated for the removal of pollutants that cannot be measured adequately or cannot be measured at all.

Perhaps the analytical chemist, as a citizen and a professional, is to blame for not getting involved and assuming a leading role in environmental problem solving. A majority of analytical chemists, especially in government and educational institutions, are "technique oriented" and simply ignore to devote their intellectual energies to urgent problems of our time. In spite of its obvious necessity, over-specialization in analytical techniques insures the inability to follow a problem through from start to finish and thereby cripples the individual. There is a pressing need for the "problem oriented" analytical chemist who can effectively participate in pollution problem-solving teams. This is a specialist in the characterization of water or air pollution. He should have the background, through



formal training or practical experience, to speak the language and to participate in the planning and implementation of pollution control programs. The analytical mind that has guided the analytical chemist to an exciting and productive career is particularly suited to undertake this challenge.

One may ask, "How adequate are present capabilities for measurement of water pollution?" and "what are the areas which need improvement?" An attempt to answer these questions is given in this presentation. This includes a brief review of the ever broadening analytical needs for water pollution control with a focus on "problem areas."

## II. The Aquatic Environment

Water quality characteristics of aquatic environments are the net result of a multitude of physical, chemical and biological interactions. Rivers, lakes, estuaries are continuously under a dynamic state of change with respect to their geological age and geochemical characteristics. This is manifested by a continuous circulation, transformation, and accumulation of energy and matter through the medium of living things and their activities. A multiplicity of natural regulatory mechanisms play the important role of controlling the physicochemical properties of the aqueous phase and number and type of its biological population.

Through man's exploitation of his water resources the dynamic balance in aquatic ecosystems is frequently disturbed which may result in such dramatic responses as fishkill, foaming, taste and odor, *etc.* These ecological responses are described in terms of "pollution" since they interfere with man's use of the water resources.

Consequently, measurements of water quality in aquatic environments should take into account physicochemical and biochemical interactions between the hydrosphere and biosphere as well as interactions with the atmosphere and the lithosphere (*e.g.*, bottom sediments). To this extent the characterization of water quality in rivers, lakes, *etc.* may require the measurement of the quantities and rates of mass and energy transfer across environmental boundaries, *e.g.*, CO<sub>2</sub> or O<sub>2</sub> transfer at the air-water interface and the accumulation or release of chemicals by bottom sediments.

Examples of such environmental interactions are given in Figure 1, which illustrates dynamics and the relative proportions of major elements in an aquatic ecosystem [1]. The ratios given in Figure 1 are based on the number of atoms per atom of phosphorus. The model suggests a basic

phenomenon that phosphorus, nitrogen and carbon levels are largely dependent on the synthesis or decomposition of organic matter by the aquatic biota [1].

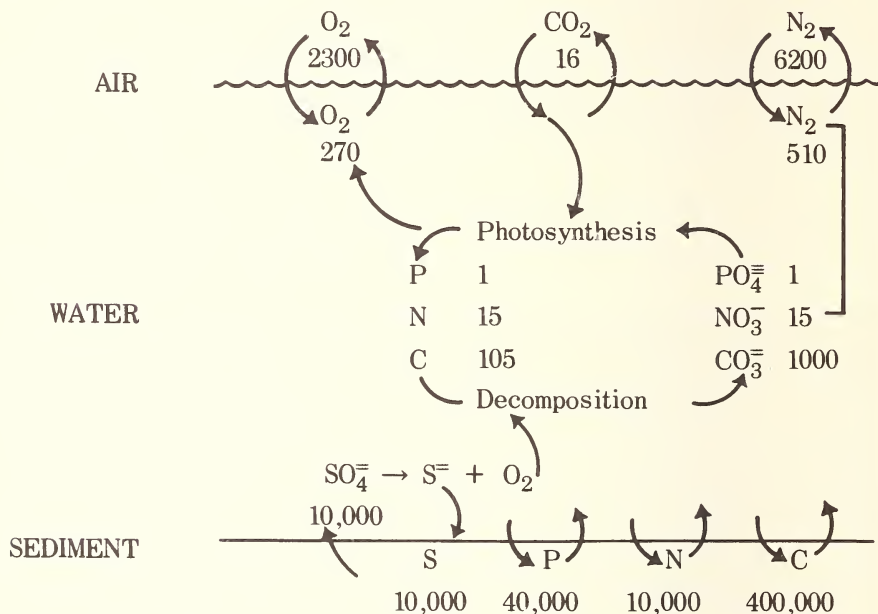


Figure 1. Interaction of some major elements in aquatic ecosystems.

The physicochemical characteristics of the aqueous phase have a direct effect on the types and distribution of the aquatic biota. The reverse is also true, the physicochemical characteristics of the aqueous phase are greatly influenced by the activity of the aquatic biota. These interactions can be easily illustrated by considering a case of a thermally stratified lake as shown in Figure 2. Certain lakes and marine environments undergo seasonal thermal stratification into a warm surface layer (epilimnion), an underlying layer where temperature drops rapidly with depth (thermocline) and a bottom deep layer of cool water of higher density (hypolimnion).

The hypolimnetic zone represents a condition where the biological decomposition of organic matter has completely removed the dissolved oxygen which cannot be replenished since these layers are stagnant and are cut off from the atmosphere. As a result of the disappearance of dissolved oxygen, anaerobic biological populations take over and start to

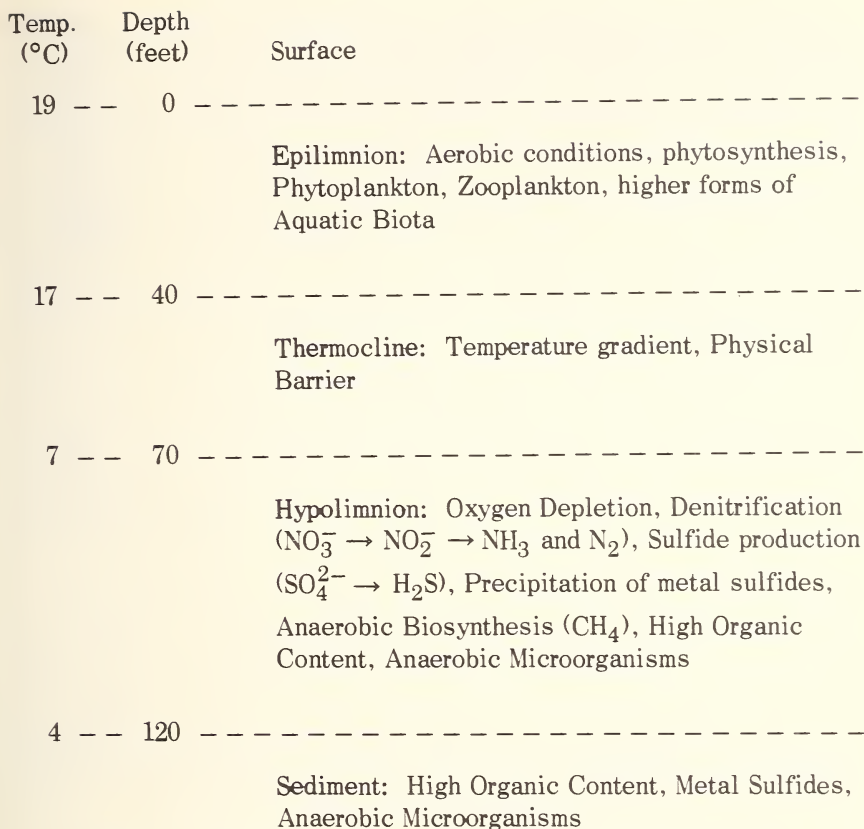


Figure 2. Thermal stratification physical-chemical-biological interactions.

reduce nitrates to nitrites and ammonia. After the nitrates and nitrites supply is exhausted, sulfate reduction takes place which results in the formation of free sulfides, *e.g.*  $\text{H}_2\text{S}$ . Besides being toxic, the sulfides will form highly insoluble metal sulfides which tend to strip the water of its heavy metal content and enrich the bottom sediments with the formed precipitates. This dramatic display of biological and chemical interactions is typical of all aquatic environments.

In view of the above discussion, full characterization of water quality for pollution control should account for (a) the distribution dynamics of chemicals in the aqueous phase (soluble, colloidal or adsorbed on particulate matter); (b) accumulation and release by the aquatic biota; (c) accumulation and release by bottom deposits; and (d) inputs from land and atmosphere *e.g.*, airborne contaminants and land run offs.

One of the more important aspects of water quality measurement is the ability to characterize the chemical species. For example, in the case of metal analysis, it is highly significant to provide analytical data in terms of the oxidation state and type of species *i.e.*, free or complexed instead of a total metal concentration. This is apparent in view of the fact that the chemical reactivity of a given metal is dependent on its form and not necessarily on its total concentration. Accordingly, the availability of metal micronutrients to the biological system or their toxicity effects are dependent on the metal species rather than the total metal concentration.

It may occur to the reader at this time that water quality characterization for pollution control is an involved expensive task which requires a lot of manpower. The fact of the matter is that in many cases only a few measurements were found to be sufficient. Like a physician dealing with a patient, the water analyst may make his diagnosis of water pollution based on few measurements. The analyst experienced in water pollution characterization can often make appropriate decisions based on practiced intuition.

### III. Design of Measurement Systems

Design of measurement systems begins with defining (a) WHY the analysis is needed (Objectives), (b) WHAT are the parameters to look for (Parameters), and (c) HOW to perform the analysis (Methods).

#### A. OBJECTIVES OF ANALYSIS

Definition of the purpose and objectives of analysis is the first step in the design of any measurement system; this includes the definition of particular problems to which solutions are sought. Some of the more common objectives of natural and wastewater analysis are as follows:

- (a) determine the suitability of a water for its intended use and to establish the degree of treatment necessary prior to its use,
- (b) estimate the possible detrimental effects of a waste effluent on the quality of the receiving water for subsequent downstream use,
- (c) evaluate necessary treatment requirements in view of water reuse,



- (d) determine the quantities of valuable by-products which could be recovered from a waste effluent,
- (e) evaluate and optimize industrial processes on a continuous or batch basis, and
- (f) provide background information on the present quality of streams and lakes which can be used to demonstrate future changes in their quality.

Natural bodies of fresh water can be classified according to intended use, including: public water supply, fish or shellfish propagation, recreation, agricultural use, industrial water supply, hydroelectric power, navigation, and disposal of sewage and industrial wastes. Water quality criteria may be based on the quality to be maintained in a receiving water, or may relate to the quality of the effluent itself. Criteria relating to the receiving water are termed "stream standards" while those relating to the waste discharge are termed "effluent standards." Both types of standards have their strengths and weaknesses.

Stream standards, which have been developed to comply with the provisions of the Water Quality Act, permit the full capacity of a stream to be utilized in the assimilation of wastes. In establishing stream standards the quality of the receiving stream is maintained at a level above that required for the desired usage. This permits full utilization of the stream's capacity to assimilate wastes and forces materials discharged into the different streams to be subjected to different degrees of treatment prior to discharge. In contrast, if effluent standards were used, all discharges of the same type to streams designated for the same use would be required to receive identical levels of treatment. The level of treatment would be consistent with the capability of advanced technology. The effect of the effluent on the receiving waters would differ and there would be no assurance that the concentration of pollutant in the stream would not exceed that critical to the desired use.

It is readily apparent that either system of standards has its flaws. It is likely that future standards will incorporate features of both stream and effluent standards.

The analysis of wastewaters which are to be discharged to municipal sewers is done principally for the purpose of evaluating compliance with certain effluent criteria set by the municipality. Effluent standards in this case are established for the purpose of protecting municipal waste treatment plants from operational interference which might be caused by industrial waste discharged and for protection of the sewer structure from damage. Both the municipality and the industry may carry out periodic

analysis of the waste effluent for purposes of control and assessment of charges, which are usually related to the strength and volume of a particular waste. It is important to point out that wastewater which is discharged to municipal sewers usually becomes the responsibility of the municipality.

An important objective of many surveys of lakes and streams is to provide a base of information to be used to evaluate future changes in water quality. Such data have been used by Beeton [2] and others in demonstrating the eutrophication of the Great Lakes. These data are important sources for other investigators. The U.S. Geological Survey has compiled data on the quality and quantity of water of surface waters of the United States and has publications on the quality of surface water for irrigation and of municipal water supply for industrial use [3,4]. Other water quality data has been published by the Public Health Service and Federal Water Pollution Control Administration [5].

## B. PARAMETERS FOR ANALYSIS

After defining the objectives of analysis, the next step in the design of measurement systems is to decide on particular constituents for which analyses are to be made and what methods are to be employed. The analyst experienced in water quality characterization can often make the proper decision based on practiced intuition. In most cases, however, certain rather well defined guidelines should be followed.

Depending on the intended use of a receiving water, the parameters listed in Table 1 are of significance for water quality characterization, and this should serve as guidelines for analyses of water quality for purposes of treatment and control [6].

The choice of parameters for analysis depends primarily on the type of information sought. Certain tests are frequently used for the identification of various types of pollution associated with industrial wastewaters. For example, Table 2 lists a number of tests and their significance.

Some of the important and most frequently used tests in the analysis of water are the nonspecific tests listed in Table 3. These tests often measure a property of a group of substances as in alkalinity where the capacity of the water to neutralize hydrogen ions is measured or a physical parameter such as density or a physiological property such as odor. Many of these tests are used to determine the suitability of natural waters for industrial or municipal use and to determine the type and degree of treatment needed to render them acceptable.

Table 1. Parameters for water quality characterization

## A. Domestic Water Supplies

Quality parameter	Permissible criteria	Desirable criteria
1. Color (Co—Pt scale)	75 units	< 10 units
Odor	Virtually absent	Virtually absent
Taste	Virtually absent	Virtually absent
2. Turbidity	— —	Virtually absent
3. Inorganic chemicals		
pH	6.0 — 8.5	6.0 — 8.5
Alkalinity (CaCO <sub>3</sub> units)	30 — 500 mg/l	30 — 500 mg/l
Ammonia	0.5	< 0.01
Arsenic	0.05	absent
Barium	1.0	— —
Boron	1.0	
Cadmium	0.01	
Chlorides	250	< 25
Chromium (hexavalent)	0.05	absent
Copper	1.0	Virtually absent
Dissolved oxygen	> 4.0	Air saturation
Fluorides	0.8 to 1.7 mg/l	1.0 mg/l
Iron (filtrable)	< 0.3	Virtually absent
Lead	< 0.05	Absent
Manganese (filtrable)	< 0.05	Absent
Nitrates plus nitrites (as mg/lN)	< 10	Virtually absent
Phosphorus	10 — 50 $\mu$ g/l	10 $\mu$ g/l
Selenium	0.01	Absent
Silver	0.05	— —
Sulfates	250	< 50
Total dissolved solids	500	< 200
Uranyl ion	5	Absent
Zinc	5.5	Virtually absent
4. Organic chemicals		
Carbon chloroform extract (CCE)	0.15	< 0.04
Methylene blue active substances	0.5	Virtually absent
5. Pesticides:		
Aldrin	0.017	— —
Chlordane	0.003	— —
DDT	0.042	— —
Dieldrin	0.017	— —

Table 1. (continued)

Quality parameters	Permissible criteria	Desirable criteria
5. Pesticides: (continued)*		
Dieldrin	0.017	--
Endrin	0.001	--
Heptachlor	0.018	--
Heptachlor expoxide	0.018	--
Lindane	0.056	--
Methoxychlor	0.035	--
Organic phosphates plus carbamates	0.1	--
Taxophane	0.005	--
Herbicides 2,4,D plus 2,4,5-T, plus 2,4,5-TP	0.1	--
6. Radioactivity		
Gross beta	1,000 pc/l	< 100 pc/l
Radium 226	3 pc/l	< 1 pc/l
Strontium-90	10 pc/l	< 2 pc/l

### B. Recreation and Aesthetics

The general requirements are that surface waters should be capable of supporting life forms of aesthetic and recreational values. Hence, surface waters should be free from

- materials that may settle to form objectionable deposits or float on the surface as debris, oil and scum,
- substances that may impart taste, odor, color or turbidity,
- toxic substances including radionuclides physiologically harmful to man, fish or other aquatic plants or animals and
- substances which may result in promoting the growth of undesirable aquatic life.

Presently, there are no well defined water quality criteria for recreation or aesthetic purposes.

### C. Aquatic Life, Fish and Wildlife

#### 1. Turbidity:

Discharge of waste in receiving waters should not cause change in turbidity in the order of 50 Jackson units in warm-water streams, 25 Jackson units in warm-lakes, and 10 Jackson units in cold-water streams and lakes.

#### 2. Color and Transparency:

Optimum light requirements for photosynthesis should be at least 10 percent of incident light on the surface.



Table 1. (continued)

## C. Aquatic Life, Fish and Wildlife (continued)

## 3. Settleable Matter:

Minor deposits of settleable matter may inhibit growth of flora and biota of water body. Such materials should not be discharged in surface waters.

## 4. Floating Matter:

All foreign floating matter should not be discharged in surface waters. A typical pollution problem is that of oil waste discharges which may result in the formation of

- a. visible objectionable color film on the surface,
- b. alter taste and odor of water,
- c. coat banks and bottoms of water course,
- d. taint aquatic biota and
- e. cause toxicity to fish and man.

## 5. Dissolved Matter:

The effect of dissolved matter on aquatic biota can be due to toxicity at relatively low concentrations or due to osmotic effects at relatively high concentrations. In general, total dissolved matter should not exceed 50 millimoles (the equivalent of 1500 mg/l NaCl).

## 6. pH, Alkalinity and Acidity

The pH range of 6.0 to 9.0 is considered desirable. Discharge of waste effluents should not lower the receiving water alkalinity to less than 20 mg/l.

## 7. Temperature:

Heat should not be added to a receiving water in excess of the amount that will raise the temperature by 3–5 °F. In general, normal daily and seasonable temperature variations should be maintained.

## 8. Dissolved Oxygen:

It is generally required to maintain a dissolved oxygen level above 4 to 5 mg/l. In cold water bodies it is recommended to maintain the dissolved oxygen above 7 mg/l.

## 9. Plant Nutrients:

Organic waste effluents such as sewage, food processing, canning and industrial wastes containing nutrients, vitamins, trace elements, and growth stimulants should be carefully controlled. It is important not to disturb the naturally occurring ratio of nitrogen (nitrates and ammonia) to total phosphorus in the receiving water.

## 10. Toxic Matter:

Waste effluents containing chemicals with unknown toxicity characteristics should be tested and proven to be harmless in the concentration to be found in the receiving waters. Discharging pesticides in natural waters should be avoided if possible or kept below 1/100 of the 48-hrs.  $TL_m$  values. Levels of ABS and LAS

Table 1. (continued)

## C. Aquatic Life, Fish and Wildlife (continued)

## 10. Toxic Matter: (continued)

should not exceed 1.0 mg/l and 0.2 mg/l, respectively, for periods of exposures exceeding 24 hours.

It should be noticed that the presence of two or more toxic agents in the receiving water may exert an additive effect.

## 11. Radionuclides:

No radionuclides should be discharged in natural waters to produce concentrations greater than those specified by the USPHS Drinking Water Standards.

## D. Agricultural Water Use

## 1. Total Dissolved Solids or "Salinity"

This is the most important water quality consideration since it controls the availability of water to the plant through osmotic pressure regulating mechanisms. The effect of salinity on plant growth varies from one type to another and is dependent on environmental conditions.

## 2. Trace Elements Tolerance for Irrigation Waters may be Summarized as Follows:

Element	Continuous water use mg/l	Short-term water use, fine texture soil mg/l
Aluminum	1.0	20.0
Arsenic	1.0	10.0
Berillium	0.5	1.0
Boron	0.75	2.0
Cadmium	0.005	0.05
Chromium	5.0	20.0
Cobalt	0.2	10.0
Copper	0.2	5.0
Lead	5.0	20.0
Lithium	5.0	5.0
Manganese	2.0	20.0
Molybdenum	0.005	0.05
Nickel	0.5	2.0
Selenium	0.05	0.05
Vanadium	10.0	10.0
Zinc	5.0	10.0

Table 1. (continued)

## D. Agricultural Water Use (continued)

## 3. pH, Acidity and Alkalinity

pH is not greatly significant and waters with pH values from 4.5 to 9.0 should not present problems. Highly acidic or alkaline waters can induce adverse effects on plant growth.

## 4. Chlorides

Depending upon environmental conditions, crops and irrigation management practices, approximately 700 mg/l chlorides is permissible in irrigation waters.

## 5. Temperature

Very high as well as very low temperatures of irrigation waters can interfere with plant growth. Temperature tolerance is highly dependent on the type of plant and other environmental conditions.

## 6. Pesticide

A variety of herbicides, insecticides, fungicides and rodenticides can be present in irrigation waters at concentrations which may be detrimental to crops, livestock, wildlife and man. As far as effect on plant growth and permissible levels are concerned, these are variable and highly dependent on the type of chemical, type of plant, environmental factors and exposure time.

## 7. Suspended Solids

Suspended solids in irrigation waters may deposit on soil surface and produce a crust which inhibit water infiltration and seedling emergence. In waters used for sprinkler irrigation colloids and suspended matter may form a film on leaf surface which impair photosynthesis and defer growth.

## 8. Radionuclides

USPH Drinking Water Standards are usually applied to irrigation waters.

Some of the more frequently measured parameters in pollution studies are listed in Table 4. The specific chemical analyses to be performed in a water pollution study will ultimately depend on the types of materials discharged and on the desired uses of the receiving water.

## C. METHODS OF ANALYSIS

Following the establishment of the objectives of the measurement program, and the selection of parameters for analyses, suitable analytical

Table 2. Significance of parametric measurements.

Test or determination	Significance
Dissolved solids	Soluble salts may affect aquatic life or future use of water for domestic or agricultural purposes.
Ammonia, nitrites, nitrates, and total organic nitrogen	Degree of stabilization (oxidation) or organic nitrogenous matter.
Metals	Toxic pollution.
Cyanide	Toxic pollution.
Phenols	Toxic pollution, odor, and taste.
Sulfides	Toxic pollution, odor.
Sulfates	May affect corrosion of concrete, possible biochemical reduction to sulfides.
Calcium and magnesium	Hardness.
Synthetic detergents	Froth, toxic pollution.

Table 3. Non-specific water quality parameters.

Physical parameters	Chemical parameters	Physiological parameters
Filterable residues	Hardness	Taste
Salinity	Alkalinity and acidity	Odor
Density	Biochemical oxygen demand (BOD)	Color
Electrical conductance	Chemical oxygen demand (COD)	Suspended matter
	Total carbon	Turbidity
	Chlorine demand	



Table 4. Tests used for the measurement of pollution of natural waters.

Nutrient demand	Specific nutrients	Nuisances	Toxicity
Dissolved oxygen	Nitrogen:	Sulfide	Cyanide
Biochemical oxygen demand	Ammonia	Sulfite	Heavy metals
Chemical oxygen demand	Nitrate	Grease and oil	Pesticides
Total carbon	Nitrite	Detergents	
	Organic nitrogen	Phenols	
	Phosphorus:		
	Orthophosphate		
	Polyphosphate		
	Organic phosphorus		

methods are then selected. There are no prescribed procedures which are applicable to all situations but the best method for any given situation must be based upon consideration of many factors. Some of the more important factors are: (a) required sensitivity, (b) accuracy of method, (c) presence of interferences, (d) number of samples to be analyzed, (e) necessity of field or *in situ* analyses, (f) speed required for results, (g) availability of required instruments, (h) number and skill of laboratory personnel, and (i) required use of standard or referee methods.

Another point for consideration in selecting analytical methods concerns the collection, transportation and storage of samples. Screening tests should be conducted for purposes of approximating required sample volumes, establishing desirable sites for and frequency of sampling, and providing a rough estimate of the waste composition and strength.

Listings of "standard" and "recommended" methods for analysis of natural waters and wastewaters are to be found in a variety of publications sponsored by several water works, pollution control, and public health agencies and organizations in this country and abroad. In addition, in several instances certain private industries have found it desirable to formalize listings of more specific methods for analysis of particular types of industrial wastewater.

While general procedures of analysis for specific waste constituents are highly useful, the analyst must be careful to guard against overreliance

upon such procedures, and against the possibility of being lulled into a false sense of security by results obtained from application of such procedures in instances where they may not be applicable. Indiscriminate application of general-purpose methods for analysis without due consideration of specific interferences and other problems must be avoided.

#### D. INTENSIVE *Versus* EXTENSIVE MEASUREMENT

Physical and chemical characterization of water quality can be categorized conveniently as intensive *versus* extensive measurements. This categorization should not be considered in terms of rigorous thermodynamic entities but rather in terms of conceptual quantitative properties of the system under investigation. It is for reasons of convenience, which will become apparent later, that we should differentiate between intensive and extensive water quality parameters.

In textbook terminology extensive properties are additive in the sense that the total value of a system is the sum of the individual values for each of its constituent parts. Conversely, intensive properties are not additive, and can be specified for any system without reference to the size of that system. This is illustrated in Table 5.

The chemical potential or the molar free energy change  $(\partial G/\partial n)_{T,P}$  is further defined:

$$\mu - \mu^0 = RT \ln a \quad (1)$$

Table 5. Extensive and intensive parameters.

System designation	Intensive parameter	Extensive parameter	Work done by the system
Gravitational	Height ( $h$ )	Mass ( $m$ )	$= mg(h_2 - h_1)$
Thermal	Temperature ( $T$ )	Heat capacity ( $C_p$ )	$= C_p(T_2 - T_1)$
Electrical	Voltage ( $E$ )	Charge ( $q$ )	$= q(E_2 - E_1)$
Chemical	Chemical potential ( $\mu$ )	Number of moles ( $n$ )	$= n(\mu_2 - \mu_1)$

where  $h$  = height, cm

$g$  = acceleration, cm/s<sup>2</sup>

$m$  = mass, g

$C_p$  = heat capacity, cal/deg mol

$T$  = temperature, °C

$\mu$  = the chemical potential, cal/mol

$E$  = voltage, V

$n$  = number of moles

$q$  = charge, C

where  $a$  is the activity given in a molar scale and  $\mu^\circ$  is the chemical potential at a reference state. The activity can be related empirically to concentration  $C$  by the equation:

$$a = \gamma C \quad (2)$$

where  $\gamma$  is the activity coefficient. Accordingly, the activity,  $a$ , is an intensive parameter and is a direct measure of the difference between the chemical potential in the actual and in the reference state.

In defining a chemical system it is important to distinguish between intensive properties based on chemical potential measurements and extensive properties based on counting the number of moles of a given substance. This can easily be illustrated in comparing data from potentiometric measurements of pH, pX or pM where X and M refer to anions and cations respectively, with those from titrimetric determinations of acidity, anions or cations. In the former case measurement is based on potential determinations which are essentially intensive parameters while in the latter case measurement is based on stoichiometric calculation. Results of analysis of either type may not agree particularly if interferences are present which may cause the activity coefficient to deviate from unity, *e.g.*, salting-in or salting-out agents.

Similarly, in the case of dissolved oxygen voltammetric membrane electrode systems the measured parameter is essentially an intensive factor, since the diffusion current is solely dependent on the difference in the chemical potentials of molecular oxygen across the membrane. The diffusion current equation at steady state conditions is as follows:

$$i_d = \left[ zFA P_m \frac{1}{b} \right] a_{O_2} \quad (3)$$

where  $i_d$  is the diffusion current,  $z$  is the number of electrons transferred,  $F$  is the Faraday,  $A$  is the cathode surface area,  $P_m$  is the membrane permeability coefficient,  $b$  is the membrane thickness and  $a_{O_2}$  is the activity of molecular oxygen.

Dissolved oxygen measurement by the membrane electrode (ME) does not have to be equal to results obtained by titration methods, such as the Winkler test. In the former case, the measured parameter is the activity of molecular oxygen, while in the latter case, the total number of oxygen molecules in the titrated sample is estimated by stoichiometric calculations. This difference can be easily realized on applying both the membrane electrode and the Winkler test to water samples containing salting-in and salting-out agents, as shown in Figure 3.

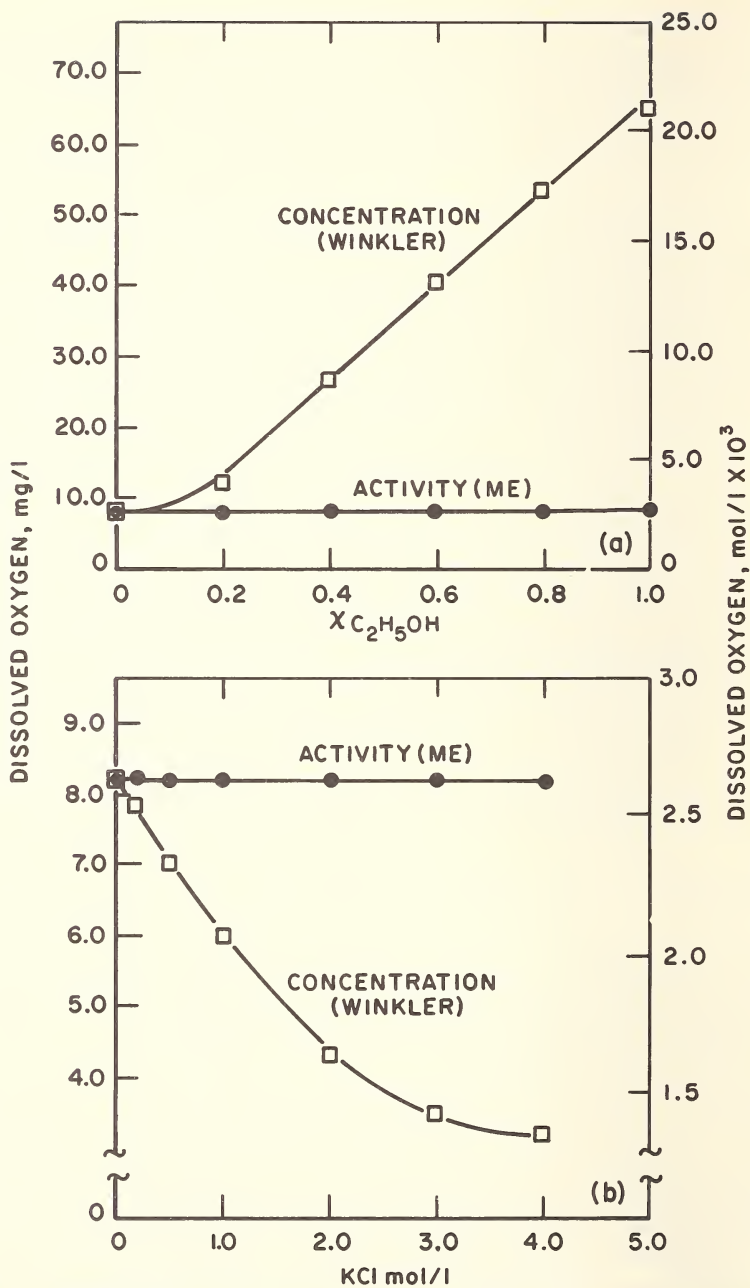


Figure 3. Effects of (a) salting-in and (b) salting-out.  $P_{O_2} = 0.21$  atmosphere, temperature  $= 25 \pm 0.5^\circ C$ .



Results shown in Figure 3 were obtained with water samples containing varying concentrations of potassium chloride and ethyl alcohol and were equilibrated with one atmosphere of air ( $P_{O_2} = 0.21$  atm) at constant temperature. Being a sparingly soluble gas, oxygen solubility in water follows Henry's Law and the following expression applies:

$$P_{O_2} = H a_{O_2} = H \gamma_{O_2} C_{O_2} \quad (4)$$

where  $H$  is Henry's Law constant. Consequently, the test solutions had the same activity and varying concentrations of dissolved oxygen depending on the type and concentration of the salting-in or salting-out agents. The results in Figure 3 show that the membrane electrode gave identical diffusion current values indicating that the test solutions had the same oxygen activity. In the meantime in the presence of KCl, the salting-out effect caused a decrease in oxygen solubility with increasing salt concentrations as shown by the results from the Winkler titration. In this case the activity coefficient,  $\gamma_{O_2}$ , increased with increasing ionic strength according to the following relationship:

$$\ln \gamma_{O_2} = K_s I \quad (5)$$

where  $K_s$  is the salting-out coefficient and  $I$  is the ionic strength.

Conversely, in the presence of ethyl alcohol, the salting-in effect caused an increase in oxygen solubility with increasing alcohol concentration as shown by results from the Winkler titration. Under these conditions the activity coefficient,  $\gamma_{O_2}$ , decreased with increasing alcohol concentration.

The above example may serve to explain the controversy in measurement of dissolved oxygen in natural and wastewaters as well as in biological fluids. This is exemplified by the present confusion in expressing dissolved measurement in terms of oxygen tension, percent saturation, partial pressure, *etc.* Activity measurement, *per se*, is considered most significant in characterizing biochemical and physicochemical transformations in aquatic environments. For example, oxygen transfer across the air-water interface or within the bulk of the aqueous phase under constant temperature and hydrodynamic conditions is solely dependent on the gradient in activity, and not always in the direction of diminishing concentration. Thus, under certain conditions, "uphill" diffusion, counteracting equalization of concentration may occur. Similarly, in biological systems the availability of molecular oxygen for biochemical reactions depends primarily on the activity level and hence respiration rates are more precisely described in terms of oxygen activity than in terms of concentra-

tions. This distinction is significant in the characterization of waste effluents, rivers, lakes and estuaries, although in many applications it may be possible to assume that the activity coefficient is close to unity and the difference between activity and concentration is negligible for all practical purposes.

### E. SAMPLING

The significance of a chemical analysis is no greater than that of the sampling program. Ideally speaking, a representative sample does not exist. Attempts are made however to come as close as possible to sample aquatic environment without disturbing its physicochemical and biochemical characteristics. There is no universal procedure for sampling applicable to all kinds of natural and wastewaters. The analyst has to design his sampling program based on a complete understanding of the purpose of analysis and the type of parameters to look for.

The most important requirements for a satisfactory sample are that it be both valid and representative. For a sample to be valid, it has to be one which has been collected by a process of random selection. Random selection is one of the most basic, yet most frequently violated, principles in development of a sampling program. Any method of sampling that sacrifices random selection will impair statistical evaluation of the analytical data. If nonrandom sampling procedures are contemplated—perhaps for significant reasons of convenience—it is highly desirable to first demonstrate that the results of the analysis check those which would be obtained by random sampling. This check would be essential prior to any statistical evaluation of the data.

A satisfactory sample is not only randomly drawn, but also is representative. This means that the composition of the sample should be identical to that of the water from which it was collected; the collected sample should have the same physicochemical characteristics as the sampled water at the time and site of sampling.

Planning for a sampling program should be guided by the overall objectives of analysis. Major factors of concern for any sampling program are: (a) frequency of sample collection, (b) total number of samples, (c) size of each sample, (d) sites of sample collection, (e) method of sample collection, (f) data to be collected with each sample, and (g) transportation and care of samples prior to analysis.

Frequency of sampling will depend to a large extent upon the frequency of variations in composition of the water to be sampled. There are two

principal types of sampling procedures commonly used for analysis of natural and wastewaters. The first type is that which yields instantaneous spot or grab samples, while the second type yields integrated continuous or composite samples. A grab sample is a discrete portion of a wastewater taken at a given time; a series of grab samples reflects variations in constituents over a period of time. The size of such individual samples will depend on the objectives and methods of analysis, and on the required accuracy. The total number of grab samples should satisfy the statistical requirements of the sampling program.

Composite samples are useful for determining average conditions, which when correlated with flow can be used for computing the material balance of a stream of wastewater over a period of time. A composite sample is essentially a weighted series of grab samples, the volume of each being proportional to the rate of flow of the waste stream at the time and site of sample collection. Samples may be composited over any time period such as 4, 8, or 24 hours, depending on the purposes of analysis.

Selection of sampling sites should be made with great care. A field survey is often useful in planning for site selection. In the case of sampling of a stream, special consideration should be given to sources of waste discharge, dilution by tributaries, and changes in surrounding topography [7,8].

Sampling of wastewater from pipes or conduits is more complicated than stream sampling, especially when the water to be sampled is under pressure. For example, in the case of a chemical treatment plant, selection of sampling sites may require extensive investigations and preliminary checking of samples from a number of effluent outlets. Proper positioning of the sampling outlet within the cross section of a conduit is essential for obtaining a representative sample, particularly for conduits of large diameter. The choice of a sampling site within the cross section of a conduit is best done by examining and comparing samples drawn from several points along the vertical and horizontal diameters of the conduit. The cross-sectional area of the opening or inlet of the sampling line should be such that the flow of water in this line is proportional to the flow of the water in the conduit. An elaborate discussion on sampling of water from pipes and conduits can be found in the ASTM Manual on Industrial Water and Industrial Waste Water [9].

Wastes discharged by industry are of great variety, and sampling must be tailored to suit the particular characteristics of a given wastewater. Sampling procedures can be expected to vary widely from one wastewater to another. Special procedures have been reported for use with water sampled under reduced or elevated pressure and/or temperature



[10]. Procedures and equipment used for the sampling of waters containing dissolved gases and volatile constituents susceptible to loss upon aeration have been described [10].

A certain amount of precaution is sometimes required in sampling processes for reasons of safety. For example, strict precautionary measures [11] should be followed in taking samples from deep manholes to guard against accumulation of toxic and explosive gases and insufficiency of oxygen.

Sampling can be accomplished by either manual or automatic means, again depending on the purpose of analysis and method of sampling. A grab sample is usually collected manually. When it is necessary to extend sampling over a considerable period of time, or when a continuous (repetitive) record of analysis at a given sampling point is required, automatic sampling equipment is commonly used.

Continuous sampling equipment, correctly designed and installed, will provide more frequent samples, tend to eliminate human errors, and in many cases be economically more feasible. A variety of automatic sampling equipment suitable for water sampling under variable conditions and for different purposes, is presently available [10].

The maintenance of complete records regarding the source of the sample and the conditions under which it has been collected is an inherent part of a good sampling program. This is of particular importance in field, river, or in-plant surveys, where a great number of samples are collected from different sources and under variable conditions. For illustrative purposes, the U.S. Geological Survey has defined the minimum data required for samples of surface and groundwaters [11] (see p. 319).

One of the most important aspects of the sampling process is the care and preservation of the sample prior to analysis. This point cannot be overemphasized. A water analysis is of limited value if the sample has undergone physicochemical or biochemical changes during transportation or storage. These changes are time dependent, but they usually proceed slowly. In general, the shorter the time that elapses between collection of a sample and its analysis, the more reliable will be the analytical results. Certain constituents may, however, require immediate analysis at the sample site.

Certain determinations are more sensitive than others to the method of handling of water samples before analysis. Changes in temperature and pressure may result in the escape of certain gaseous constituents (*e.g.*, O<sub>2</sub>, CO<sub>2</sub>, H<sub>2</sub>S, Cl<sub>2</sub>, CH<sub>4</sub>), or the dissolution of some atmospheric gases (*e.g.*, O<sub>2</sub>). It is recommended, therefore, that determinations for gases be done in the field, or, to "fix" such materials as O<sub>2</sub>, Cl<sub>2</sub> and H<sub>2</sub>S, the sample



## Minimum data requirements for water samples.

## Surface Waters

Name of water body  
 Location of station or site  
 Point of collection  
 Date of collection  
 Time of collection  
 Gage height or water discharge  
 Temperature of the water  
 Name of collector, and  
 Weather and other natural or  
 other man-made factors  
 that may assist in interpreting  
 the chemical quality

## Ground Waters

Geographical and legal locations  
 Depth of well  
 Diameter of well  
 Length of casing and positions of  
 screens  
 Method of collection  
 Point of collection  
 Water bearing formation(s)  
 Water level  
 Yield of well in normal operations  
 Water temperature  
 Principal use of water  
 Name of collector  
 Date of collection  
 Appearance at time of collection, and  
 Weather or other natural of man-made  
 factors that may assist in  
 interpreting chemical quality

should be treated upon collection with stable oxidizing or reducing agents. It is also recommended that the temperature and pH of the water be determined at the site of sampling. Changes in temperature and pH may cause changes in the solubility of dissolved gases and certain nonvolatile constituents, resulting in their separation from aqueous phase. Carbonic acid-bicarbonate-carbonate equilibria may be shifted to release gaseous  $\text{CO}_2$ , or to precipitate certain metal carbonates. Similarly, shifts in hydrogen sulfide-sulfide equilibria due to changes in pH and/or temperature may result in the escape of  $\text{H}_2\text{S}$  or the precipitation of metal sulfides.

Heavy metals ions may undergo a variety of physicochemical transformations during sample handling. It has been recommended that for analyses for Al, Cr, Cu, Fe, Mn and Zn, samples should be filtered at the site of collection and acidified to about pH 3.5 with glacial acetic acid [11]. Acidification tends to minimize precipitation, as well as sorption on the wall of the sample container. Since acetic acid may stimulate growth

of molds, it may be necessary to add a small quantity of formaldehyde to the sample solution as a preservative.

Another major point of interest for handling water samples is the effect of biological activity on the sample characteristics. Microbiological activity may be effective in changing the nitrate-nitrite-ammonia balance, in reducing sulfate to sulfide, in decreasing the dissolved oxygen content, biochemical oxygen demand (BOD), organophosphorous compounds and any readily degraded organic compound. Freezing of water samples is helpful in minimizing changes due to biological activity. Certain chemical preservatives, such as chloroform or formaldehyde, are sometimes added to water samples for this purpose.

That it is practically impossible to handle and process a water sample without changing its characteristics should be recognized. The best chance for error-free procedure lies in the use of *in situ* analyses. In the end, the dependability of even a well planned sampling program rests upon the experience and good judgment of the analyst.

#### IV. Water Quality Surveillance Programs

Pollution control activities rely heavily on the ability to monitor and survey the quality of natural and wastewaters. This includes rivers, lakes, estuaries, as well as industrial and domestic waste effluents. Presently, there are several state and federal water surveillance programs designed to (a) acquire, evaluate and disseminate information on the quality of waters for and from the varied local, state, interstate and federal agencies together with educational, commercial, industrial and individual entities, (b) determine the long term trends and variations of water quality, and (c) provide a rapid intelligence system for the preservation of the waters and the protection of the water users, including compliance with water quality standards.

Water quality surveillance activities may be based on manual surveillance, aerial surveillance, public surveillance and automatic surveillance techniques.

##### A. MANUAL SURVEILLANCE

Manual surveillance is based on the periodic collection of water samples, their transportation and storage and subsequent laboratory analysis.

Sampling sites and frequency are selected to give an overall evaluation of water quality in drainage basins and effects of waste discharge.

### B. PUBLIC SURVEILLANCE

In an attempt to increase the scope of the overall surveillance program certain states [12] initiated public surveillance programs, sometimes referred to as the "water watchers." Similar to traffic safety patrols for school children, citizen groups are encouraged to watch for the safety of water resources and to telephone and report abnormal conditions of streams or lakes, *e.g.*, oil or visually apparent chemical spills and fishkills.

### C. AERIAL SURVEILLANCE

Photography and remote sensing from aeroplanes are being used to gather information on (a) materials on the water surface, *e.g.*, oil spills, (b) suspended matter in the water, and (c) certain soluble compounds. Measurement is based on the interactions of electromagnetic energy with matter floating, suspended or in solution. The electromagnetic energy source may be the sun, in which case sensors depending on solar reflections are referred to as "passive" systems, or the energy source may be an "active" source, *e.g.*, laser [13]. Applications of "passive" airborne systems, the measurements in the ultraviolet, visible and infrared regions of the electromagnetic spectrum have been recently reported in the literature [13-15].

A number of chemical pollutants can be detected by this technique based on their fluorescence properties. This includes a variety of compounds such as chlorophyll, phenol, ligninin-sulfonates, Rodamine WT and oils. Fluorometric techniques seem to be more applicable to airborne remote sensing than absorption techniques. Beside being more sensitive than absorption techniques, fluorometric procedures were found to be less affected by turbidity and quite useful in the analysis of mixtures [15]. As low as 5 ppb of Rodamine WT were detected using airborne fluorometric measurement [15]. In this case, the sun was used as the excitation source; hence, the procedure was limited to daylight hours.

Remote multispectral scanning techniques have been used for the measurement of various pertinent water quality parameters. Temperature measurement are usually made in the 8 - 13.5  $\mu\text{m}$  region throughout the

day and night to an accuracy of  $0.5^{\circ}\text{C}$  [13]. This was used advantageously in thermal pollution measurement which provided the ability of a complete instantaneous mapping of large water areas.

Detection of oil spills can be easily done with airborne remote sensing using IR and UV multispectral scanning [14]. This is considered to be a very powerful technique because of its ability to follow the movement of the oil slicks over large areas of water surface.

The University of Michigan Willow Run Research Laboratory developed a unique multispectral system [13,14] with the ability to examine a scene in 17 bands distributed between 0.3 and 1.35  $\mu\text{m}$  (*i.e.*, a 17 channel spectrometer with electronic processing capabilities). This system was used for aerial surveying of oil spills and industrial waste discharge in lakes, rivers, *etc.* The system is capable of automatically determining the optimum wavelength, and in addition, the interactions of radiation with the effluent at other positions in the electromagnetic spectrum are also determined. Each effluent type and receiving body of water has its unique spectral characteristics which are used to differentiate it from other sources.

Aerial surveillance by multispectral remote sensing techniques seems to have a great potential in pollution monitoring. Such monitoring systems may not be able to provide information on the chemical composition of the system under investigation. Nevertheless, it is considered to be a valuable tool in the study of the dynamic conditions in aquatic environments and large scale water resources investigations.

#### D. AUTOMATED SURVEILLANCE SYSTEMS

Automated surveillance systems rely on the use of automatic, unattended measurement procedures operated on a continuous basis or intermittently at a determined frequency. Automatic water quality monitoring systems are being widely used in this country and abroad in a variety of water quality management programs [12,16-18]. The main advantages in using automated systems lie in the ability of maintaining a continuous, instantaneous record on water quality in rivers, lakes and estuaries, industrial effluents, *etc.* Changes in water quality may occur suddenly as a result of intense storms, industrial spills, *etc.* In certain cases changes in water quality undergo rapid variation such as dissolved oxygen and salinity changes from natural tidal fluctuations.

Manual surveillance techniques seem to be inadequate and certainly not practical to monitor sudden changes in water quality.



The main objectives of automatic water quality monitoring activity are to (a) detect violations of standards or other undesirable quality conditions so that remedial action can be taken quickly, (b) the establishment of water quality baseline and trends, (c) verify and provide data for predictive calculations on water quality including computerized mathematical models for the purpose of establishing design and operational criteria for water pollution control facilities, and (d) determine short-range water quality trends for program management and public information purposes. Selection of monitoring sites is usually made at places where (a) rapid fluctuations in water quality or quantity, (b) high potential for accidental spills of wastes, and (c) hydrologic or other conditions which permit continuous evaluation of the quality response of the stream system to waste discharges and other pollutional impacts.

### *1. System Characteristics*

There are two main approaches for water quality measurement in automatic monitoring systems. The first approach is based on *in situ* measurement by means of electrochemical transducers without altering the physicochemical characteristics of the test solution. The second approach is based on the use of automated repetitive wet analytical procedures in which chemicals are added to the test solution and measurements are made after suitable chemical reactions have taken place, *e.g.*, the Technicon AutoAnalyzer.

Monitoring systems in which the measurement is done by transducer systems (without the addition of reagents) are being used widely for monitoring water quality of rivers, streams, estuaries and industrial and domestic waste effluents. These systems are usually capable of measuring temperature, electrical conductivity, dissolved oxygen, turbidity, pH, sunlight intensity, chlorides, oxidation-reduction potential (ORP) and alpha and beta radioactivity. Certain parameters are measured for specific applications, *e.g.*, the monitoring of fluoride ions, residual chlorine, nitrates and hardness in drinking water supplies [19] and monitoring cyanides, sulfides, copper ions in certain waste effluents.

Electrochemical sensors used for water quality monitoring systems can be categorized based on the type of measurement, *i.e.*, (a) conductometric, (b) potentiometric, and (c) voltammetric sensors. Table 6 lists examples of sensor systems used in water quality monitors and the underlying basic relationship.

Table 6. Electrochemical sensors.

## Type

- a. Conductometric 
$$L = K_e \sum_i^n C_i \lambda_i z_i$$
- b. Potentiometric 
$$E_m = \text{constant} + \frac{RT}{z_i F} \ln \left[ a_i + K_j a_j^{z_i/z_j} \right]$$
1. Glass electrode 
$$\text{pH} = -\log a_{\text{H}^+}$$
2. Inert metal electrode (redox potential) 
$$\text{pE} = -\log a_{\text{H}} / (2.3 RTF^{-1})$$
  
 Cationic 
$$= \text{pM}^+ = -\log a_{\text{M}^+}$$
3. Potentiometric membrane electrodes 
$$\text{Anionic} = \text{pA}^- = -\log a_{\text{A}^-}$$
- c. Voltammetric membrane electrodes (dissolved oxygen) 
$$i_d = \left[ z F A P_m \frac{1}{b} \right] a_{\text{O}_2}$$

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$L$  = specific conductance

$K_c$  = cell constant

$C_i$  = ionic concentration

$\lambda_i$  = ionic equivalent conductance

$z_i$  = ion valency

$E_m$  = measured electrode potential

$F$  = the Faraday constant

$K_j$  = selectivity coefficient

$i_d$  = diffusion current

$A$  = electrode surface area

$P_m$  = membrane permeability coefficient

$b$  = membrane thickness

Electrical conductivity is a gross estimate of the ionic strength of the test solution. It is dependent on the ionic concentration  $c_i$  (number), equivalent ionic conductance  $\lambda_i$ , ionic charge  $z_i$  and water viscosity. Conductance measurement cannot be used as an indication of the concentration unless all other variables in the conductance equation are held constant. Consequently, equality in electrical conductance of two different waters may not mean equality in the total dissolved solids.

Attempts are being made to incorporate the newly developed potentiometric membrane electrodes (selective ion electrodes) in water quality monitoring systems [19]. Table 7 gives a listing of commercially available electrodes with some of their properties [20]. It should be noted that the measured potential is a function of the activity of free ions and these electrodes are insensitive to complexed ionic species. This is one of the main advantages of these systems since this property can be used to differentiate between free and complexed metallic species.

Table 7. Potentiometric membrane electrode.

Electrode system	pH range	Principal interference
Bromide	0 - 14	$\text{CN}^-$ , $\text{I}^-$ , $\text{S}^{2-}$
Cadmium	1 - 14	$\text{Ag}^+$ , $\text{Hg}^{2+}$ , $\text{Cu}^{2+}$ , $\text{Fe}^{2+}$ , $\text{Pb}^{2+}$
Calcium	5.5 - 11	$\text{Zn}^{2+}$ , $\text{Fe}^{2+}$ , $\text{Pb}^{2+}$ , $\text{Cu}^{2+}$ , $\text{Ni}^{2+}$
Chloride	0 - 14	$\text{Br}^-$ , $\text{I}^-$ , $\text{S}^{2-}$ , $\text{CN}^-$ , $\text{SCN}^-$ , $\text{NH}_3$
Cyanide	0 - 14	$\text{S}^{2-}$ , $\text{I}^-$
Cupric	0 - 14	$\text{Ag}^+$ , $\text{Hg}^{2+}$ , $\text{Fe}^{3+}$
Fluoride	0 - 8.5	$\text{OH}^-$
Iodide	0 - 14	$\text{S}^{2-}$ , $\text{CN}^-$
Lead	2 - 14	$\text{Ag}^+$ , $\text{Hg}^{2+}$ , $\text{Cu}^{2+}$ , $\text{Cd}^{2+}$ , $\text{Fe}^{2+}$
Nitrate	2 - 12	$\text{I}^-$ , $\text{Br}^-$ , $\text{S}^{2-}$ , $\text{NO}_2^-$ , $\text{CN}^-$ , $\text{HCO}_3^-$ , $\text{Cl}^-$ , $\text{OAc}^-$ , $\text{CO}_3^{2-}$ , $\text{SO}_3^{2-}$ , $\text{S O}_3^{2-}$
Sulfide	0 - 14	
Hardness	5.5 - 11	$\text{Zn}^{2+}$ , $\text{Fe}^{2+}$ , $\text{Cu}^{2+}$ , $\text{Ni}^{2+}$ , $\text{Ba}^{2+}$ , $\text{Sr}^{2+}$

In cases where kinetics of interactions between free and combined ions are fast, equilibrium relationships can be used in conjunction with Nernst equation to measure free, combined and total species concentration [21]. For example, free sulfides  $[S^{2-}]$  alone may be measured by the membrane electrode. But if the pH is known, the following equilibrium relationships can be used to calculate  $[HS^-]$ ,  $[H_2S]$  and total sulfides  $[S_T]$ ,

$$\log [S_T] = \log [S^{2-}] + \log \left[ \frac{[H^+]^2}{K_1 K_2} + \frac{[H^+]}{K_1} + 1 \right] \quad (6)$$

$$\log [H_2S] = \log [S^{2-}] + \log \frac{[H^+]^2}{K_1 K_2} \quad (7)$$

$$\log [HS^-] = \log [S^{2-}] + \log \frac{[H^+]}{K_1} \quad (8)$$

Voltammetric membrane electrodes for dissolved oxygen measurement of the galvanic or polarographic types are widely used in water quality monitoring systems [22,23]. The electrode diffusion current is linearly proportional to the activity of dissolved oxygen in the test solution. This relationship is dependent on the membrane thickness " $b$ " and permeability coefficient " $P_m$ ."

In monitoring systems the most critical part is the sensor and the reliability of measurement is usually dependent on the reliability of the sensor system. This is true whether the sensor is an electrode, a thermistor or a photoelectric cell. A clear understanding of the operation characteristics of the sensor and its dynamic response is essential. This is based on proper calibration, servicing, maintenance and alertness for small clues that may indicate malfunction.

Primary sensor characteristics are defined in terms of (a) sensitivity, (b) response time, (c) selectivity, (d) long term stability, (e) accuracy, and (f) precision. Secondary sensor characteristics are those which define the environmental effects, *e.g.*, (a) temperature, (b) flow, (c) ionic strength, (d) pH, (e) sunlight, *etc.*

### Primary Sensor Characteristics:

Sensitivity is usually defined in terms of the smallest change in the measured variable that causes a detectable change in the indication of the instrument. It specifies the lower limit of detection of the sensor. Sensitivity is directly proportional to the slope of the curve relating the signal magnitude to the amount of detectable material present. This will reflect directly on the ability to ascertain a difference between the signal and



background noise at the detection limit, *i.e.*, given adequate precision, the greater the sensitivity, the better the detectability.

The limit of detection of analytical method is the lowest concentration whose signal can be distinguished from the blank signal. This value depends on the sensitivity of the method, as well as the signal-to-noise ratio required to discern the response due to a sample. Advances in electronics have brought about the design of instruments with greater inherent stability and, therefore, lower limits of detection. Use of an on-line digital computer in fast-sweep derivative polarography has permitted the resolution of closely spaced peaks and extended the analytical sensitivity of the technique by more than an order of magnitude.

The speed of the sensor response to changes in the test solution is referred to as the "response time." It is an indication of the time needed for the sensor signal to follow 90, 95 or 99 percent of instantaneous full scale change in the measured variable. The response time should be specified for each sensor indicating whether it is dynamic or static sensor response.

Selectivity of the sensor refers to the effect of interferences resulting from detectable ions or molecules other than the species of interest. Since all sensor systems cannot achieve absolute or 100 percent selectivity, then it is important to specify the selectivity limitations in a given test solution. If the type and amount of the interfering species are known, then it is possible to incorporate the term "selectivity coefficient" in the sensors sensitivity expression. Also, in certain cases it is possible to incorporate interferences effects in the sensor calibration curve. This can be done by means of the standard addition technique where known amounts of the measured ions are added to the test solution and the proportional signal values are recorded.

Long term stability usually refers to the change in the sensor's performance characteristics with time. This is used to decide on the frequency of checking the calibration or servicing the sensor. Long term stability is a property of the particular system and is dependent on the presence of interferences and the physicochemical characteristics of the test solution.

Deviations of results by a given sensor from the "true" value define the accuracy of the system. If the source of error is found, and it is possible to correct for, this is called "determinate error." If the deviation from the true value is compounded indiscriminately by many small errors, it is simply a "random error." Random errors are subject to statistical treatment of the data.

Precision is defined in terms of the reproducibility of the sensor measurement. The more scatter in successive readings, the less precise are the

measurements. Usually, precision is closely identified with random errors and statistical theories.

An important distinction between precision and accuracy is that accurate measurements are always precise, but the converse is not necessarily true. The precision of a series of measurements may be good, but every result may be higher than the true value because of an unsuspected interference. Only when determinate errors are minimized will precision also imply accuracy.

### Secondary Sensor Characteristics:

Secondary sensor characteristics refer to the effect of environmental variables. This can be a result of changes in the sensors primary characteristics or changes in the physiochemical characteristics of the test solution. For example, temperature effects on conductance measurement are quite complex since the temperature coefficient is dependent on both ionic strength and temperature. The conductivity of sea water was found to increase by 3 percent per degree increase in temperature at 0 °C, 2 percent increase at 25 °C and about 5 percent increase at 30 °C. It is therefore advisable to measure relative conductance rather than absolute values [24]. This is done by measuring the ratio of the conductance of the test solution to that of a reference solution at the same temperature. Thermistors or resistances can be used instead of the reference solution.

It is always advisable to establish the primary and secondary sensor characteristics for each sensor independently before using it for field applications. Not only these characteristics will vary from one sensor type to another, but also differences between two sensors of the same type and from the same manufacturer may occur.

The second main type of monitoring systems is based on automating wet analytical techniques, *e.g.*, the Technicon AutoAnalyzer. Such systems are essentially capable of automatic sampling, filtering, diluting, reagent addition, mixing, heating, digesting, and after appropriate delay for color development, colorimetric measurement are done. All these steps, which are usually done by an analyst, are automated and performed on a stream of samples moved by a fixed speed peristaltic pump.

This technique finds its widest application in laboratory operations where large samples of waters are handled daily. There have been certain attempts however to use this technique for water quality monitoring where the AutoAnalyzer is kept in a trailer on a river bank [25]. In other applications autoanalyzers were used for monitoring silicates and nitrates in sea water off the coast of California [26], and for monitoring nutrients concentrations in Lake Erie [27].

Table 8. Parameters measured by the Technicon CSM 6.<sup>a</sup>

Parameter	Nominal range <sup>b</sup> (ppm)	Detection limit (ppm)
Phosphate	0-8	0.08
Chromium (hexavalent)	0-5	0.05
Copper	0-10	0.10
Iron	0-10	0.10
Ammonia	0-10	0.10
Methyl orange alkalinity <sup>c</sup>	0-500	5.0
Thymol blue alkalinity <sup>c</sup>	0-160	1.0
Hardness <sup>c</sup>	0-300	3.0
Sulfate <sup>d</sup>	0-500	5.0
Phenol	0-5	0.05
Cyanide <sup>c</sup>	0-3	0.03
Chemical oxygen demand	0-500	5.0
Chloride <sup>c</sup>	0-10	0.10
Nitrite	0-1.5	0.015
Nitrite + nitrate	0-2.0	0.02
Fluoride <sup>c</sup>	0-2.5	0.025
Orthophosphate	0-10	0.10
Silicate	0-15	0.15

<sup>a</sup> From reference 25<sup>b</sup> Alternate ranges optional<sup>c</sup> Deviates from linearity<sup>d</sup> Sulfate is only available on continuous monitoring applications

One of the main advantages of applying the "AutoAnalyzer" for water quality monitoring purposes lies in its ability for measurement of a large number of parameters. Table 8 shows a listing of water quality parameters that can be measured by such systems [25].

The application of the "AutoAnalyzer" for water quality monitoring purposes has been faced with a number of limitations; (a) the effect of turbidity on colorimetric measurements, (b) these systems need more

frequent attendance for servicing, and (c) AutoAnalyzer systems seem to have a higher capital cost and running expenses in comparison to the transducer-type monitoring systems.

## *2. Data Acquisition*

Automatic surveillance systems generate large volumes of data. Careful planning in data management is essential to the successful operation of these systems.

The planning for data management should be based on the need for the data, the frequency with which the data should be collected, the optimum locations of collection, the provisions for data storage and the resources for analysis of data.

Depending on the size of the monitoring systems, a variety of computer programs have been developed to control the operation of the system and for data handling [28-31]. Computer programs to control the operation of the system are designed to: interrogate each monitor, collect the data, check for abnormal operating conditions in the monitor, compute for any unusual rates of change of the incoming data, transmit functional commands to the monitors, display alert and alarm conditions on the remote terminal, and allow human intervention when desired. In view of the large volume of data generated by these systems, it is essential to use computers for data reduction, storage and retrieval.

Computer programs permit data to be retrieved in the form of individual values by date and time of collection or summarized in the form of central tendency dispersion (maximum, minimum, arithmetic mean and standard deviation) and percentiles for each location or a group of locations. Computerized annual summaries are printed routinely for widespread distribution to water users and other interested persons [28,31].

## *3. System Appraisal*

Effective implementation of water pollution control programs rely heavily on adequate automatic water quality monitoring. Measurement by manual systems is limited by the frequency with which samples can be collected for analysis. The time delay between sampling and analysis can result in certain changes in the sample characteristics causing it to be less representative of the body of water from which it was collected. The time involved in making and reporting the analysis is time lost before any water pollution corrective action can take place. In addition, based on the availability and cost of labor, automatic monitoring systems are considered to be the least costly system.



As stated earlier, the limiting step in automatic water quality monitoring systems is the sensor system. There are only few sensor systems available at the present time which are used for measurement of a limited number of parameters. There is a pressing need for the development of sensor systems for parameters such as phosphates, trace metals (Pb, Cu, Cd, As, Zn, Hg, *etc.*) and trace organic matter (pesticides, carcinogens, phenols, *etc.*).

The reliability of a sensor system is determined by its performance characteristics under field conditions and the ability to compensate for environmental variables such as temperature, hydrostatic pressure and hydrodynamic effects. These sensor systems should also exhibit a certain amount of long term stability, in order to minimize the frequency of servicing and checking the monitor.

It is also recommended to initiate research programs for adapting recently developed sensor systems for water quality monitoring purposes. A typical example is the incorporation of "selective ion electrodes" in present water quality monitors which would significantly increase their measurement capabilities. Attempts should be made to utilize *in situ* separation and concentration techniques to increase the sensitivity and selectivity of certain sensor systems, *e.g.*, voltammetric membrane electrodes and *in situ* anodic stripping voltammetry.

## V. Analytical Methodology

Analytical procedures for measurement of water quality characteristics have been arbitrarily classified according to (a) the purpose of analysis (*e.g.*, biodegradability and toxicity tests), (b) the nature of the constituent under test (*e.g.*, gases, alkali metals, and heavy metals), and (c) the nature of the analytical procedure itself (*e.g.*, titrimetric, gravimetric, and electrometric). In this presentation, methods of analyses are classified according to the analytical information sought and whether it pertains primarily to the physical, chemical or biological characteristics of the test solution. The discussion includes a brief assessment of present measurement capabilities and future needs.

### A. PHYSICAL CHARACTERISTICS

Physical water quality characteristics of major significance are (a) density and viscosity, (b) surface tension, (c) temperature, (d) electrical conductance, (e) particulate, volatile and dissolved matter, and (f) radioactivity.

Adequate analytical procedures are presently available for measurement of the above physical characteristics. One of the physical parameters which is frequently neglected in water quality measurement programs is surface tension. Measurement of surface tension can provide a very sensitive indication of the presence of invisible surface films of oil or surface active material. The main reason for not using surface tension measurement is perhaps due to the absence of applicable monitoring procedures for surface tension.

## B. PHYSIOLOGICAL CHARACTERISTICS

Physiological characteristics of water quality include (a) color, (b) odor, (c) taste, and (d) turbidity. These parameters represent human physiological responses to physicochemical characteristics of water quality. Consequently they are considered to be most difficult to measure and in certain cases, cannot be expressed quantitatively. This is particularly true whenever human optical or olfactory senses are used to measure color or odor of water samples.

### *1. Color*

Color is a physiological response to physical optics. Until recently color measurement has been based on visual comparison of the color of water samples with standard colored solutions (*e.g.*, platinum-cobalt color solutions) or colored glass discs.

Color determinations by visual comparison are subject to a number of interferences and variables. The main drawback to this method is the subjectivity and variation in response of different individuals to color. It is obvious also that certain waters may have colors which cannot be matched well by the standard platinum-cobalt scale.

A more accurate determination of color can be accomplished by application of the tristimulus colorimetry technique in which color can be expressed in terms which approximately describe the visual response of an individual. One of these terms relates to the brightness and the degree of saturation (pastel, pale, *etc.*) by **purity**. Luminosity and purity are usually reported in units of percent, and the dominant wavelength in nanometers.

Tristimulus parameters are commonly determined from measurements of the light transmission characteristics of a filtered water sample. Trans-

mission data are converted to color classification terms by using standards adopted by the International Commission on Illumination [32]. Chromaticity diagrams are used to describe the color numerically in terms of the tristimulus parameters [33].

## 2. Odor

Odor is a human physiological response to odorant volatile matter that stimulates smell in man.

Determination of odor is based solely on the olfactory senses of the analyst, or on those of a group of individuals, and on the ability of the analyst (or group) to distinguish between different levels and kinds of odors. The testing is based entirely on arbitrary comparison since no absolute units or base for odor exist [33,34].

Several authors have attempted to characterize and classify the origin of odor in natural and wastewaters [34-39]. Most of these studies treat taste and odor as closely connected human responses. Taste determinations using human subjects are generally not recommended especially in cases of wastewater or untreated industrial effluents, and thus are excluded from the present discussion.

Odor can always be related to the presence of volatile organic and/or inorganic species present in water. Odor intensity is a function of the volatility and the concentration of the odor-causing species, as well as of certain environmental factors such as temperature, ionic strength and pressure. It has been claimed that there are only four basic types of odor: (a) sweet, (b) sour, (c) burnt, and (d) goaty, realizing that the many odors are in fact combinations of two or more of these groups.

Odors often can be related to the presence of certain biological forms in the wastewater, such as algae and actinomycetes. Such odor-causing organisms are believed to secrete characteristic volatile oils during growth, and upon decomposition and decay. Such poetic terms as musty, earthy, woody, moldy, swampy, grassy, fishy, and wet-leaves have been used to describe odors [41,42].

Recent studies of odor characteristics and human response have led to a proposal of a stereochemical theory of odor [35,41]. This theory relates the response to odor to the geometry of molecules. It has been postulated that the olfactory system is composed of receptor cells of certain different types, each representing a distinct "primary" odor, and that odorous molecules produce their effects by fitting closely into "receptor sites" on

these cells. This concept is similar to the "lock and key" theory used to explain certain biochemical reactions; *e.g.*, enzyme with substrate, antibody with antigen, and desoxyribonucleic acid with ribonucleic acid in protein synthesis.

Seven primary odors are distinguished [44], each of them by an appropriately shaped receptor at the olfactory nerve endings. The primary odors, together with reasonably familiar examples are (a) camphoraceous, *e.g.*, camphor or moth repellent, (b) musky, *e.g.*, pentadecanolactone as in angelica root oil, (c) floral, *e.g.*, phenylethyl methyl ethyl carbinol as in roses, (d) pepperminty, *e.g.*, methone as in mint candy, (e) pungent, *e.g.*, acetic acid as in vinegar, and (g) putrid, *e.g.*, butyl mercaptan as in rotten eggs. A classification of odor by chemical type is shown in Table 9 [36]. It has been claimed that every known odor can be made by mixing the seven primary odors in certain combinations and proportions [36].

Odors resulting from mixtures of two or more odoriferous substances are extremely complex. The mixture may produce an odor of greater or lesser intensity than might be expected from summing the individual odors, or a completely different kind of odor may be produced [35-37]. Accordingly, it is frequently necessary to characterize the odor of the wastewater and that of the receiving stream both separately and in combination if the actual relationship and effect are to be determined.

Odor intensity is expressed in terms of the **threshold odor number** [34,35]. By definition, the threshold odor number is the greatest dilution of the sample that still leaves a perceptible residual odor. The test procedure is based on successive dilution of a sample with odor-free water, disregarding any suspended matter or immiscible substances, until a dilution is obtained which has a barely perceptible odor. It has been recommended that odor tests be run at 25 °C and 60 °C [33] or 40 °C and 60 °C [34]. In all cases the sampling and test temperature should be reported, since the threshold odor is a function of temperature. A given sample, under fixed conditions, will emit a characteristic odor stimulus, but the response to this stimulus and the judgment based upon this response are purely subjective matters, and their interpretation may vary considerably from individual to individual [35,38,39]. Consequently, it is desirable to use a panel or group of judges, rather than a single analyst for both qualitative and semiquantitative evaluation of odors in water or wastewater samples [33].

Quantitative measurement of odor causing matter seems to be the only exact approach for odor measurement. Gas chromatographic procedures have been used to identify and measure the quantities of odors causing substances in water samples [41]. A positive identification is accom-



Table 9. Odors classified by chemical types.

Odor class	Chemical types included	Odor characteristics			Algae and fungi
		Fragrance	Acidity	Burntness	
Estery	Ethers	high	medium	low to	-----
	Esters			medium	
Alcoholic	Lower ketones	high	medium	low to	Asterionella Coelosphaerium
	Phenols and cresols			high	
	Alcohols			high	
Carbonyl	Hydrocarbons	medium	medium	low to	Malleomonas
	Aldehydes			medium	
Acidic	Higher ketones	medium	very	low to	Anabaena
	Acid anhydrides			medium	
	Organic acids			low to	
	Sulfur dioxide			medium	
Halide	Quinones	High	medium	medium	Dinobryon Actinomycetes
	Oxides (including ozone)			to high	
	Halides			high	
Sulfury	Nitrogen compounds	medium	medium	very	Aphanizomenon
	Selenium compounds			high	
	Arsenicals			high	
	Mercaptans			high	
	Sulfides and hydrogen sulfide			high	
Unsaturated	Acetylene derivatives	high	medium	medium	Synura
	Butadiene			high	
	Isoprene			high	
Basic	Vinyl monomers	high	medium	low to	Uroloporopsis Dinobryon
	Higher amines			medium	
	Alkaloids			medium	
	Ammonia and lower amines				

plished once a correlation is established between the isolated substance and its human odor sensation. Additional research work is needed to reduce these techniques to a practical level for day to day operations and automatic measurement procedure applicable for monitoring purposes.

### 3. Turbidity

The term turbidity is commonly used to signify a visual response to the absorption and scatter of light by suspended matter in a given water. Accordingly, turbidity represents a physiological response to physical optics similar to color.

Turbidity is measured in terms of the amount of light scattered and/or the light absorbed by matter suspended in the water. The classic procedure for turbidity measurement is based on the preparation of a series of standard suspensions and comparison with the test solution using a Duboscq type comparator.

Measurements are usually done in reference to a standard suspension of fine silica [33]. The standard procedure for the measurement of turbidity [34] is based on the use of "Jackson Candle Turbidimeter." The test is based on measuring that length of light path through the solution at which the outline of the flame of a standard candle becomes indistinct. Results are arbitrarily reported in "Jackson Turbidity Units." This is done in reference to a standard silica suspension, or a formazin polymer suspension which is easier to prepare than the silica suspension.

The turbidity of a given water is a function of both the amount of light absorbed and light scattered by the sample. Light scattered by suspended solids depends on the number, size, shape and refractive index of the particles as well as on the wavelength of the exciting light. If the number of particles in suspension is small, particles act as independent scatterers and the measured scatter is proportional to the particle concentration. At higher particle concentrations the light scattered is rescattered and inter-particle interference occurs.

Turbidity measurement is usually made by light absorption and light scatter techniques. Light absorption measurements are usually applied to water with high turbidity.

The term "optical turbidity,  $T$ " is frequently used [43] as a measure of turbidity in terms of light scattered by a test solution in a given cell light path. This is defined as follows:

$$I = I_0 e^{-Tb} \quad (9)$$

or

$$\log (I_0/I) \frac{2.3}{b} = A \frac{2.3}{b} = T \quad (10)$$

where  $I_0$  and  $I$  are intensities of incident and transmitted light and  $b$  is length of light path. Light scatter measurements are usually made at  $90^\circ$  to the incident light beam with a precision in the order of 1 percent.

Waters of turbidity in excess of 1000 Jackson units are diluted prior to measurement. For waters of low turbidity (less than 25 Jackson turbidity units) light scattering techniques are most commonly used [33,34]. Black and Hannah [43] have discussed the theoretical and procedural aspects of turbidity measurements with the Jackson Candle method and more sophisticated methods. A simple low-angle photometer that may be calibrated with clay suspensions in terms of Jackson turbidity units is described and recommended for use with low turbidity waters. Several commercial turbidity monitoring systems are available, and have found wide use for monitoring water quality [44].

New developments in turbidity measurement should stress the following areas (a) the effect of color of the test solution, (b) instruments capable of measurement over a wide range of turbidity, and (c) a correlation between turbidity and human visual responses.

### C. ANALYSIS FOR METALS

#### *1. Significance*

Metal analysis for water pollution control is usually concerned with (a) alkaline earths (hardness metals, *e.g.*, Ca and Mg), (b) iron and manganese, and (c) trace metals (*e.g.*, Cu, Cd, Pb, Zn, Cr, Ni, Mo, Hg, and As). The most challenging aspect in metal analysis is considered to be species characterization at the concentration levels present in the aquatic environment. This is particularly true with trace metals.

Metals such as Cu, Cd, Pb, Zn, Cr, Ni, Hg, and Mo are frequently found in the aquatic environment in the part per billion range ( $10^{-8}M$  to  $10^{-10}M$ ). Some of these metals play a significant role in biologically mediated reactions in natural waters. In trace quantities, many of these metal ions serve as essential micronutrients for enzymatically mediated transformations, but in high concentrations they may exert inhibitory or

toxic effects on biological systems. Lead and mercury, in particular, are toxic to biological systems and are considered to be direct signs of man-made pollution.

It has been generally assumed that the availability of metal micronutrients and the toxicity of metals to biological systems are solely dependent on metal concentration. This has been the basis for establishing tolerance limits and water quality standards.

This assumption is not quite valid, since the availability of metal micronutrients to biological systems and metal toxicity effects are highly dependent on the degree and type of metal complex formation. The biological response to trace metals in aquatic environments is greatly influenced by the presence of metal binding ligands of natural or pollutional origin and whether the metal species are in solution or in the form of colloidal particulates.

Trace metals in the aquatic environment also may serve as "markers" to provide a finger-print identification of water masses. The distribution and relative abundance of metals may offer valuable information on the geochemical history and pollutional characteristics of natural waters. Transport dynamics of metals between the hydrosphere and biosphere, lithosphere and atmosphere provide pertinent information on the geochemical cycle and the effect of man's activity.

Trace metals in public water supplies have great public health significance. It has been known that trace metals are necessary constituents in many metabolic processes in the human body. Such metals as Cu, Pb, Cd, Co, Zn and Mo have been found in the human body in trace quantities, although their function and effect are not yet known. However, the presence of excessive quantities of such metals in human water intake causes toxic effects. In spite that the normal daily metal intake from food and drinking water may not seem harmful, the human body has the capacity to concentrate some of these metals to a point where it may cause a "long-term" toxic effect.

Insofar as the water supply industry is concerned, trace metal concentration can be used as a diagnostic criterion to determine the adequacy of the water source and the safety of the distribution system. This has been particularly useful in detecting signs of certain industrial pollution of the water source and corrosion problems in the water distribution system.

In recent years there has been a strong interest in the analysis of trace metals in drinking water. This has been particularly significant in certain epidemiological studies which infer a relationship between trace metal content in drinking water and cardiovascular diseases, dental caries in school children and certain diseases of the bone.



## 2. Metals in Aquatic Environments

Metals in aquatic environments may be found in one or more of the following forms:

- a. Soluble species, (i) free hydrated metal ions and (ii) metal complexes with inorganic or organic ligands.
- b. Insoluble particulates, (i) colloidal particulates of metal complex or aggregates of hydrous metal oxides and (ii) metal complexes adsorbed or suspended particles.

A full characterization of metals in natural waters should take into account the above mentioned forms. The existence of the metal in one form or another is highly dependent on the temperature, pH, pE, and the ionic strength. Transformation from one form to another may be chemically or biochemically (enzymatic) mediated.

A schematic diagram showing metal dynamics in an environmental segment is shown in Figure 4. Inputs from the atmosphere may be in the form of aerosol or metal particulates. Metal accumulation and release from bottom sediments may occur mass transport of interstitial waters or reversible adsorption or ion exchange reactions with bottom sediments. In the aqueous phase, metals may exist as free metal, complexed with inorganic ligands, *e.g.*,  $\text{OH}^-$ ,  $\text{CO}_3^{2-}$  or complexed with organic ligands, *e.g.*, amines, proteins, vitamins, humic acid, *etc.* These forms of metals may be in solution, insoluble particulates or within the aquatic biota, *e.g.*, algae, plankton, *etc.*

The effect of biological activity on metal transformations in aquatic environments is shown in Figure 5. Mercury discharges in the form of metallic mercury  $\text{Hg}^0$ , inorganic divalent mercury  $\text{Hg}^{2+}$ , phenyl mercury  $\text{C}_6\text{H}_5\text{Hg}^+$  or alkoxy-alkyl mercury  $\text{CH}_3\text{O-CH}_2\text{-Hg}^+$ , can all be converted to the highly toxic methylmercury  $\text{CH}_3\text{Hg}^+$  or dimethyl mercury  $(\text{CH}_3)_2\text{Hg}$  in aquatic environments. The biological methylation of mercury occurs primarily in bottom sediments through the activity of certain microorganisms.

## 3. Measurement Procedures

Metal analyses have undergone significant changes in the last three decades. Prior to about 1940, most analytical techniques for metals were either gravimetric or volumetric. Since the post-war years of the late

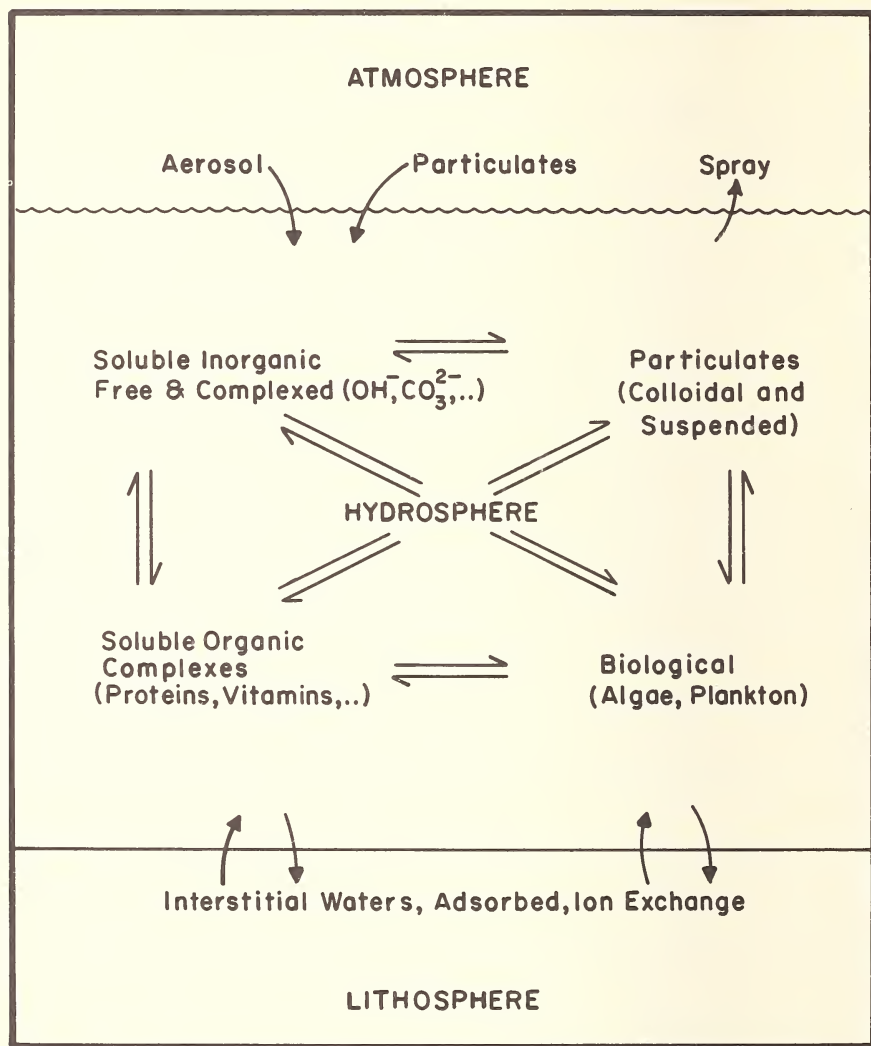


Figure 4. Metal dynamics in environmental segment.

1940's there has been a considerable increase in the use of the spectrophotometric techniques, largely as a result of the development of various organic reagents such as dithizone, o-phenanthroline, sodium diethyldithiocarbamate and diphenylcarbazide which form color-producing compounds with metal ions in solution. Many of these reagents are highly specific for particular metals and find wide application in water analysis.

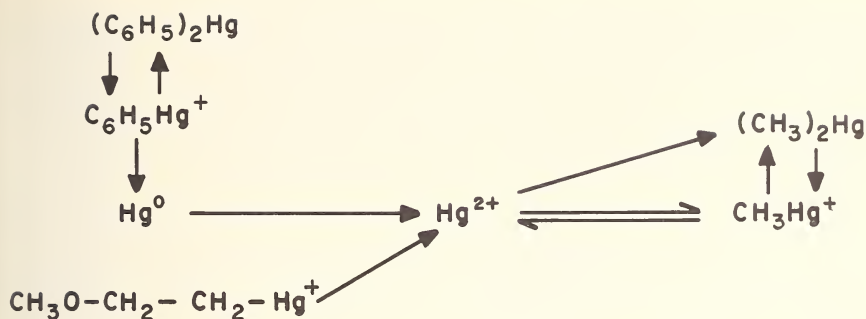


Figure 5. Biochemical transformations of mercury compounds in aquatic environments.

Compleximetric titrations with ethylene diamine tetra acetate (EDTA) and a variety of specific metal ion indicators also are used extensively for analysis of metal ions in waters and waste-waters. Perhaps the most familiar example is the analysis for calcium, magnesium and water hardness by titration with EDTA.

**a. Concentration and separation techniques.**— Various separation and concentration techniques are available for removing interferences, for extraction of colored organometallic complex compounds, or for concentration prior to titrimetric, spectrometric, electrometric, or radiometric analysis. In certain cases, the separation itself is sufficiently specific that it may be followed by a nonselective analytical procedure. In other cases, a mixture of two or more metals, ions may be separated from solution and then subjected to analysis by more selective analytical procedures.

Ion exchange techniques have been used extensively for separation of metal cations in natural and wastewaters. The total free metal ion content of a water sample can be determined by ion exchange [45-48]. The technique involves passing a sample of water through a hydrogen-form cation exchange resin and titrating the equivalent quality of  $H^+$  released with a standard base. Another aliquot may be titrated with EDTA for the hardness metals. Analyses for  $NH_4^+$ ,  $K^+$ ,  $Rb^+$  and  $Cs^+$  ions in water have been carried out by precipitation with tetraphenylboron. The precipitate is dissolved in acetone and the solution passed through a cation-exchange resin in the hydrogen form; the resulting free tetraphenylboron is then titrated [48].

Ion exchange chromatography offers an effective method for concentration and separation of ions from wastewaters. The ions are first concentrated on a suitable ion exchange column and then selectively eluted to be determined polarographically, radiometrically, spectrophotometrically

or spectrographically [49-51]. Iron, commonly found in industrial waste effluents, may be collected on cation-exchange columns as ferric iron, reduced to the ferrous state by a dilute ascorbic acid solution, and then eluted with a strong acid [47]. The selective separation of metals by ion exchange chromatography often can be markedly improved by using complexing agents.

Attempts have been made recently to use ion exchange membranes for the separation and concentration of metal ions prior to analysis [52,53]. The technique involves mounting a cation-exchange membrane, of two to five mils thickness, on the surface of an indicator electrode (usually carbon, platinum or gold) in a voltammetric system. The membrane serves as an ion exchange preconcentration matrix as well as a rigorously defined diffusion barrier for surface active or electroactive interferences present in the test solution. The exchange rate of metal ions between the test solution and membrane is accelerated by applying an appropriate e.m.f. across the membrane.

Perhaps the most interesting aspect of ion exchange membranes in this application is their suitability for *in situ* separations. Additionally, the ion exchange membrane itself can be used as a preconcentration matrix for subsequent determinations by activation analysis, emission, or absorption spectrophotometry.

The main problem associated with using cation-exchange membranes for transition and heavy metals in natural waters and wastewaters is their lack of specificity. This is particularly significant since the alkalis and alkaline earths are usually present in great excess.

Another limitation to the use of cation-exchange membranes for natural waters and wastewaters is the fact that most metals are found in the form of inorganic or organic complexes. The separation of such complexed metal ions by ion exchange is sometimes not possible unless the complex is first disrupted.

Solvent extraction is frequently used for metal separation. Valuable schemes for the separation of a large number of metal ions by successive extractions employ different complexing agents and organic solvents, *e.g.*, dithizone (diphenylthiocarbazone), oxine, cupferron, diethyldithiocarbamate, tetraphenyl arsonium salts, quaternary ammonium compounds and various chelating agents.

Selection of an appropriate solvent-extraction system depends on its specificity and its suitability for subsequent analytical procedures.

Partial freezing has been used for the concentration of Fe, Cu, Zn, Mn, Pb, Ni, Ca, Mg, and K in water samples in concentrations ranging from 0.1 to 10.0 mg/l [54]. Increasing the mixing rate up to some limiting value



increases cationic recovery. The effect of initial pH on recovery efficiency depends on the nature of the cation. Alkali metals (K, Ca and Mg) concentrate best at low pH while heavy metal cations (Pb, Ni and Cu) concentrate best under alkaline conditions [54].

**b. Analytical techniques.**— Ideally speaking, a suitable analytical procedure should have (a) high sensitivity and a low detection limit applicable to water analysis without sample pretreatment, (b) high selectivity and minimum effect of interferences commonly present in natural waters, (c) ability to characterize free and complexed metallic species, (d) *in situ* measurement, (e) portability and ruggedness for field operations, and (f) suitability for continuous monitoring purposes. Unfortunately, such an ideal analytical procedure is not presently available. Nevertheless, the analyst can rely on a number of techniques which offer some of the above advantages. A listing of some of the applicable analytical procedures and their sensitivity limits is given in Table 10.

**Atomic Absorption and Atomic Fluorescence Spectrophotometry.**— Among the optical methods, atomic absorption and atomic fluorescence spectrophotometry are frequently used for metal analysis in natural and wastewaters. These optical procedures have the unique advantage of virtual specificity. Exceptions are those few cases in which unfavorable matrix components are present in the sample solution. This is largely a result of the presence of certain compounds which combine with the metal under analysis form relatively nonvolatile compounds which do not break down in the flame. Calcium in the presence of phosphate exhibits this effect [55]. This may be remedied by sequestering the calcium ion with EDTA. Matrix effects may be minimized by separation or by adding approximately the same amount of matrix component to the standard solutions.

In contrast to flame photometry, there is very little interelement interference in atomic absorption spectrophotometry. Also, while sensitivity in flame photometry is critically dependent on flame temperature, this is not the case for atomic absorption spectrophotometry.

Over 60 elements can be determined readily by atomic absorption in the part per million range without sample pretreatment and with an accuracy of  $\pm 1$ -2 percent. This sensitivity can be vastly increased to the part per billion range by scale expansion or by extracting the metals in a non-aqueous solvent and spraying it into the flame. Microgram per liter quantities of cobalt, copper, iron, lead, nickel, and zinc have been determined in saline waters by extraction of metal complexes with ammonium pyrrolidine dithiocarbamate into methyl isobutyl ketone [56]. The use of organic solvents may alter the flame temperature, which, in contrast to

Table 10. Sensitivity limits of common analytical procedures.

Method of analysis	Sensitivity mole/l
Molecular absorption spectrophotometry	$10^{-5} - 10^{-6}$
Molecular fluorescence spectrophotometry	$10^{-7} - 10^{-8}$
Atomic absorption spectrophotometry	$10^{-6} - 10^{-7}$
Atomic fluorescence spectrophotometry	$10^{-7} - 10^{-8}$
Optical and emission spectroscopy	$10^{-5} - 10^{-6}$
Neutron activation analysis	$10^{-9} - 10^{-10}$
Potentiometry with metal, specific glass or membrane electrodes	$10^{-4} - 10^{-5}$
Classical polarography	$10^{-5} - 10^{-6}$
Derivative polarography	$10^{-6} - 10^{-7}$
Square-wave and linear-sweep voltammetry	$10^{-7} - 10^{-8}$
Anodic stripping voltammetry with hanging mercury drop electrodes	$10^{-7} - 10^{-9}$
Anodic stripping voltammetry with thin film or solid electrodes	$10^{-9} - 10^{-10}$

flame photometry, will generally have no significant effect. An increase of about 60 percent in the atomizer efficiency can be achieved with the use of certain organic solvents.

Although atomic absorption spectrophotometry is a relatively new technique, it is being applied widely for analysis of metal ions in natural waters and wastewaters. In addition to its selectivity and sensitivity, atomic absorption spectrophotometers are easy to operate and maintain.

Atomic fluorescence spectrophotometry offers no increase in selectivity over atomic absorption spectrophotometry since the latter is virtually specific for each element. It is possible, however, to increase the sensitivity of measurements with atomic fluorescence spectrophotometry by increasing the intensity of irradiation, or by increasing the amplification until the system becomes noise limited [57]. The increase in sensitivity

can be seen on comparing the light absorption and fluorescence equations. For absorption spectrophotometry, the absorbance,  $A$ , is directly related to the concentration of the absorbing species,  $C$ , the length of the light path through the absorbing solution,  $\ell$  and the molar absorptivity of the absorbing species,  $\epsilon$ ; *i.e.*,

$$A = \log (I_0/I) = \epsilon \ell C \quad (11)$$

where  $I_0$  and  $I$  are the intensities of the incident and emitted light, respectively.

The fluorescence equation may be expressed as follows:

$$F = [2.303 \phi I_0 \epsilon \ell p] C \quad (12)$$

where  $F$  is the amount of fluorescence generated,  $\phi$  is a constant related to the efficiency of fluorescence,  $I_0$  is the intensity of incident radiation,  $\epsilon$  is the molar absorptivity at a given wavelength,  $\ell$  is the pathlength in cm,  $p$  is a fractional constant, and  $C$  is the concentration. Hence,  $F$  measured in terms of the signal response of a photomultiplier tube sensitive to fluorescence radiation is proportional to the analytical concentration  $C$ , while the parameters  $I_0$ ,  $\ell$  and  $p$  are instrumental factors, and the parameters  $\phi$  and  $\epsilon$  are functions of the efficiency of the fluorescent reagent system.

It is evident from equation (11) that for absorption spectrophotometry that any increase in  $I_0$  will be accompanied by a matching increase in  $I$ , with no net gain in the absorbance,  $A$ . However, for fluorescence spectrophotometry, any increase in  $I_0$  will be matched by a corresponding increase in the analytical signal  $F$ , as indicated in equation (12). Also, any increase in the amplifier gain in absorption spectrophotometry will amplify  $I_0$  and  $I$  correspondingly, whereas in fluorescence spectrophotometry, this will result in an increase in  $F$ . For these reasons the sensitivity of fluorescence spectrophotometry is inherently greater than that of absorption spectrophotometry.

The main limitations of atomic absorption and atomic fluorescence spectrophotometry techniques are (a) inability of species characterization, (b) lack of sensitivity for direct measurement without concentrating the sample, (c) these procedures are primarily laboratory procedures unsuitable for field use, and (d) inability for *in situ* measurement.

**Activation Analysis.**—Very high sensitivity can be attained by activation analysis, which finds several applications in water pollution control activities. Sensitivities of the order of  $10^{-12}$  are obtainable with neutron

fluxes of  $10^{12} \text{ sec}^{-1} \text{ cm}^{-2}$  [58, 59]. Application of activation analysis to natural waters and wastewaters generally requires radiochemical separation of the sample and comparison of the activities of the unknown sample and of a known mass of standard treated under identical conditions.

Characterization of elements in a given water sample may be done by identifying type, energy, and half life of emission. The concentration of a given element in a sample is determined by quantizing its characteristic emission, *i.e.*,

$$\frac{\text{Mass of element X in sample}}{\text{Mass of element X in standard}} = \frac{\text{Total activity from element X in sample}}{\text{Total activity from element X in standard}}$$

Some of the factors governing quantitative measurements are: the concentration of stable elements, activation probability, neutron flux, relative abundance of stable isotopes, and time of irradiation. A wide range of separation techniques have been associated with activation analysis procedures, some of which have been automated effectively [60]. Considerable improvement in the resolution of individual peaks in spectra has been accomplished by replacement of sodium iodide detectors with germanium detectors for gamma-ray scintillation spectrometry.

Detailed procedures for activation analysis of natural waters and wastewaters have been reported [61-63]. Generally, the accuracy and precision of these procedures are about  $\pm 10$  percent. Principal errors in analysis are due primarily to self shielding, unequal flux at the sample and standard positions, inaccurate counting procedures and counting statistics. The problem of identifying the components of a complex spectrum has led to the use of computers to facilitate interpretation of available data [64]. Computers may be used also to determine optimum conditions for irradiation of particular samples. Some of the main advantages of activation analysis are: (a) its very high sensitivity, (b) the rapidity of analysis, and (c) the nondestructive nature of the test.

Although activation analysis offers the high sensitivity required for trace metal analysis, its use is limited by the availability of reactor facilities; furthermore, it is an elemental analysis procedure that offers no information about oxidation state or degree and type of metal complexation.

**Electroanalytical Methods.**—Comparatively speaking electroanalytical techniques find limited applications in water quality analysis for pollution control. This is surprising since electroanalytical techniques, which are most suitable for metal analysis, offer a number of unique advantages not shared by other methods of analysis. Generally speaking, the main advantages offered by electroanalytical procedures are (a) ability of species characterization, (b) *in situ* analysis, (c) suitability for field operation



(portability) and ruggedness), (d) high sensitivity (*e.g.*, anodic stripping voltammetry), and (e) suitability for automation and continuous monitoring purposes.

For the purpose of this discussion, electroanalytical methods are conveniently classified as being either based on the passage of faradaic current, *e.g.*, voltammetry—or based on electrode equilibrium and non-faradaic current measurement, *e.g.*, potentiometry [65]. A summary of some of the more important basic relationships underlying electroanalytical procedures is given in Table 11.

**Polarographic Techniques.**—As a result of the capacitance current used in charging the double layer, the sensitivity of classical polarography with the dropping mercury electrode is limited to approximately  $10^{-5}M$ . However, by means of preconcentration techniques it may be possible to extend the sensitivity range significantly. Copper, bismuth, lead, cadmium, and zinc have been measured in the range of 0.01 mg/liter after extraction with dithizone and carbon tetrachloride [66]. Preconcentration by ion exchange, freeze drying, evaporation, or electrodialysis may be used.

A significant problem in the application of classical polarography to natural wastewaters is the effect of interferences by electroactive and surface active impurities. Such impurities, frequently present in wastewaters, may interfere with electrode reaction processes and cause a suppression and/or a shift of the polarographic wave [67].

Modifications of polarographic techniques, such as “differential polarography” and “derivative polarography,” may be used to increase the sensitivity and minimize the effect of interferences [68]. Pulse polarography has the advantage of extending the sensitivity of determination to approximately  $10^{-8}M$ . The technique is based on the application of short potential pulses of 50 ms on either a constant or gradually increasing background voltage. Following application of the pulse, current measurements are usually done after the spike of charging current has decayed. The limiting current in pulse polarography is larger than in classical polarography. Derivative pulse polarography, which is based on superimposing the voltage pulse upon a slowly changing potential (about 1 mV/s) and recording the difference in current between successive drops *versus* the potential, is even more sensitive than pulse polarography [68].

Cathode-ray polarography or oscillographic polarography has been used for analysis of natural waters and wastewaters, with a sensitivity of  $10^{-7}M$  being reported [69-71]. This technique involves the use of a cathode-ray oscilloscope to measure the current-potential curves of applied (saw-tooth) potential rapid sweeps during the life-time of a single mercury drop. Multiple sweep techniques are also applicable. The peak

Table 11. Basic electroanalytical relationships applicable to metal analysis.

I. Methods based on faradaic current measurement

A. Transient techniques

Chronoamperometry:  $E = \text{constant}$ ,  $i = f(c, t)$

$$\text{Cottrell equation: } i_t = zFA \left[ \frac{D}{\pi t} \right]^{0.5} C$$

Chronopotentiometry:  $i = \text{constant}$ ,  $E = f(c, t)$

$$\text{Sands equation: } \tau^{0.5} = \left[ \frac{zFAD^{0.5}\pi^{0.5}}{2i} \right] C$$

Applications include pulse, square-wave, and sign-wave voltammetric techniques.

B. Steady state techniques

$$\text{Ilkovic equation (polarography): } i = \left[ kzD^{1/2}m^{2/3}t^{1/6} \right] C$$

$$\text{Voltammetric membrane electrodes: } i = \left[ zFAP \frac{1}{mb} \right] C$$

C. Modifications

Anodic stripping voltammetry (ASV)

$$\text{Plating step: } Q_t = Q_0 (1 - e^{-kt})$$

Stripping step:

Randles-Sevcik equation (HDME):

$$i_p = \left[ kz^{3/2}AD^{1/2}v^{1/2} \right] C$$

$$E_p = E_{1/2} - 1.1RT/zF$$

Roe and Toni equation (Thin film ASV):

$$i_p = \left[ zFSl \frac{\Phi}{e} v \right] C$$

$$E_p = E_0 + \frac{2.3}{\phi} \log \frac{\partial l v}{D}$$

Table 11. (continued)

## II. Methods based on non-faradaic current measurement

## Potentiometry (membrane electrodes)

$$\text{Nernst equation: } E = E_0 + \frac{RT}{zF} \ln \left[ a_i + \sum_j K_j a_j^{z_i/z_j} \right]$$

current ( $i_p$ ) in the resulting polarogram is related to the concentration of the electroactive species for a reversible reaction.

Oscillographic polarography has the advantages of: (a) relatively high sensitivity, (b) high resolution, and (c) rapidity of analysis. Traces of Cu, Pb, Zn and Mn can be determined at 0.05 mg/ml level in natural waters by this technique [71].

**Anodic Stripping Voltammetry.**—One of the most useful electrochemical approaches to metal analysis in trace quantities is anodic stripping voltammetry. This technique involves two consecutive steps: (a) the electrolytic separation and concentration of the electroactive species to form a deposit or an amalgam on the working electrode, and (b) the dissolution (stripping) of the deposit. The separation step, best known as the pre-electrolysis step, may be done quantitatively or arranged to separate a reproducible fraction of the electroactive species. This can be done by performing the pre-electrolysis step under carefully controlled conditions of potential, time of electrolysis, and hydrodynamics of the solution.

The stripping step is usually done in an unstirred solution by applying a potential—either constant or varying linearly with time—of a magnitude sufficient to drive the reverse electrolysis reactions. Quantitative determinations are done by integrating the current-time curves (coulometry at controlled potential) or by measurement of the peak current (chronoamperometry with potential sweep). Several modifications of the separation and stripping steps have been reported [72].

Hanging-drop mercury electrodes of the Gerischer's type [73] or of Kemula's type [74] have been widely used for anodic (or cathodic) stripping analysis. Greater sensitivity has been achieved by use of electrodes which consist of thin films of mercury on a substrate of either platinum, silver, nickel, or carbon [75]. Errors due to non-faradaic capacitance current components can be minimized by proper choice of stripping technique.

The main advantage of stripping voltammetry is its applicability to trace analysis. The technique has been applied for metal analyses in sea water [76], natural waters [77,78] and wastewaters [78], and drinking water supplies [79].

Anodic stripping voltammetry can be used to measure metal complex formation in aquatic environments. Metal ions in natural waters may be complexed with simple inorganic ligands such as water, halides, carbonate, and sulfate, or they may be tied up with complex organic ligands such as amino acids, organic acids, vitamins, porphyrins, humic acids, and tannins.

Qualitative and quantitative information on free and combined metal ions and metal-binding ligands and their distribution can be gained by varying the sample pretreatment and the anodic stripping procedures. A flow chart showing various analytical steps is given in Figure 6. Total metal content can be determined by digestion of the sample before anodic stripping analysis, and filtration separates dissolved from particulate fractions on the basis of variations in particle size.

In addition, it is possible to exchange the metal in many complexes by additions of acid ( $H^+$ ) or metal cations; the freed metal ions subsequently may be determined by anodic stripping voltammetry (Table 12).

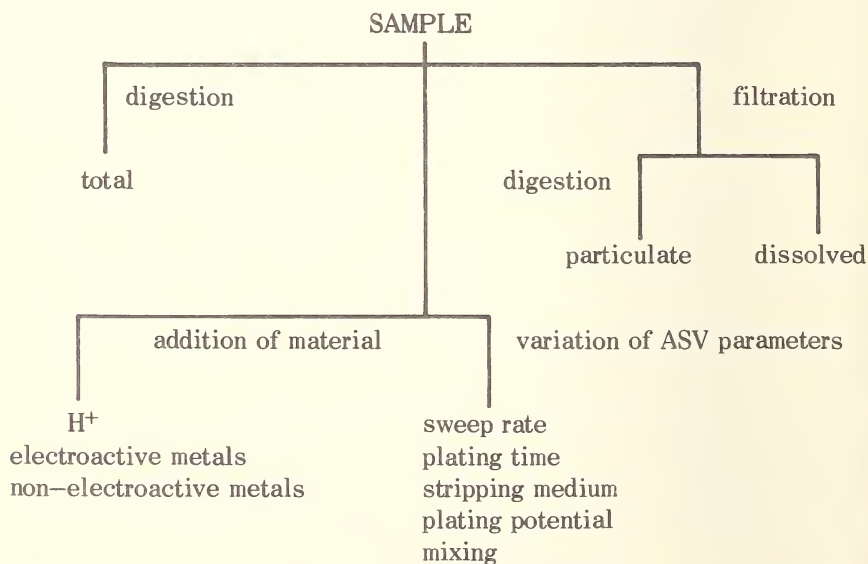


Figure 6. Analytical scheme for anodic stripping voltammetric determination of metals in natural waters.



Table 12. Trace metal concentrations in various natural and waste waters determined by anodic stripping voltammetry.

Sample	Location	Concentration ( $\mu\text{g/l}$ )				
		Free			Acid exchangeable	
		Cd	Pb	Cu	Pb	Cu
1	Rouge River 0.5 mile (0.8 km) above Ford Motor Co. turning basin	<0.1	1.1	2	15	27
2	Rouge River turning basin	7.4	2.2	14	37	108
3	Rouge River mouth	1.5	0.4	8	11	19
4	Detroit River, Trenton Channel	0.8	0.4	18	5	28
5	Detroit River, Livingston Channel	1.9	0.4	11	6	29
6	Lake Erie, central basin, surface water	—	—	—	1.8	6.8
7	Lake Erie, central basin, bottom water	0.3	0.07	0.5	1.6	1.8
8	Lake Michigan, Waukegan, Illinois	1.0	0.2	0.8	3.3	18
9	Lake Michigan, Ludington, Michigan	—	—	—	0.6	0.4

The metal-complexing ability of natural waters, which is a measurable characteristic, depends on the type and concentration of simple or complex ligands. Quantitative and qualitative information on metal-binding ligands can be obtained by a complexometric titration procedure in which an electroactive metal ion is used as the titrant. In this technique the step-wise metal-ligand complex formation can be followed and the stoichiometric end points can be detected by anodic stripping. Similarly, the rates of metal-ligand dissociation on titration with a strong acid can be determined.

Some of the more pertinent diagnostic criteria that can be drawn on using anodic stripping voltammetry [80] are (a)  $i_p$  measurement can differentiate between free metal ions and labile metal complexes on one hand and nonlabile metal complexes on the other hand, (b)  $E_p$  measurement can

differentiate between free metal ions and complexed metal compounds, (c)  $i_p$  measurement, together with  $E_p$ , can differentiate between free metal ions and labile metal complexes, and (d) metal-organic titration techniques in which electroactive metals are the titrant can differentiate between labile and nonlabile and complexes as well as the competitive interactions between metal-organics complex formation.

The analytical feasibility of anodic stripping voltammetry can be extended greatly by varying the deposition time, the potential sweep rate, the stripping medium, and the mixing regime. These variations can be used to advantage to characterize free and complexed metal ions in the aqueous environment.

**Potentiometric Techniques.**—Recently developed potentiometric membrane electrodes, known as ion selective electrodes, are extremely useful for measurement of a variety of metals in natural and wastewaters. Electrode systems are available for the measurement of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  [70]. The detection limits for these electrode systems are in the range of  $10^{-5}$  to  $10^{-6}M$ . Since  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are usually found in the concentration range of  $10^{-4}$  to  $10^{-3}M$  in natural waters, they are easily detected by ion selective electrodes [81]. Hardness measurement (divalent metal ions) is a typical example where these selective ion electrodes prove to be useful.

Selective ion electrodes cannot be applied directly for measurement of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$  in natural waters, since these metals are usually found in trace quantities  $10^{-8}M$  to  $10^{-10}M$ . They may be applied however, for measurement of heavy metals in certain industrial waste effluents such as plating industry, pickling waste and steel effluents.

Selective ion electrodes are sensitive to the activity of the electroactive species. In order to use such electrode systems for determination of concentrations rather than activities, it is important to consider the effects of ionic strength on the activity coefficient of the electroactive species and the liquid junction potential between the test solution and the reference electrode. To avoid the uncertainty of estimating an activity coefficient, it is useful to adjust the ionic strength of the sample to that of a standard solution. Since the total ionic strength of the solution determines the activity coefficient for a specific ion, the activity coefficient of the ion being analyzed in the test sample will be identical to that in the standard solution. A constant ionic strength can be obtained by using a "swamping electrolyte." This technique is frequently referred to as the "ionic medium" method. In cases where the ionic strength of the test solution does not change significantly, direct potentiometric measurement can be made. This is the case with most water supplies.

Generally speaking, the analyst is advised to determine the selectivity, sensitivity range and long term stability of the selective ion electrode before its application. This is particularly significant in applications to natural and wastewater systems where interferences may occur.

Perhaps the most significant feature of selective ion electrodes is their selectivity to free ions. This feature can be used to an advantage for the characterization of metal species. Through a sequence of potentiometric titration techniques, it is possible to construct distribution diagrams of metal species as a function of certain ligand concentrations. Such information is most valuable in the understanding of the aquatic chemistry of metals and should give added insight in the applicability of equilibrium diagrams calculated from thermodynamic constants.

Furthermore, selective ion electrodes can be used in the study of the kinetics of metal complex formation. The rates of interactions between certain metals, *e.g.*, Ca, Mg, Cu, Cd, *etc.*, and certain organic or inorganic ligands can be used to elucidate the distribution dynamics of metals in aquatic environments. Moreover, this information will offer a better understanding of the effect of discharge of certain waste effluents in natural waters.

A schematic diagram of an analytical scheme for selective ion electrode application is shown in Figure 7.

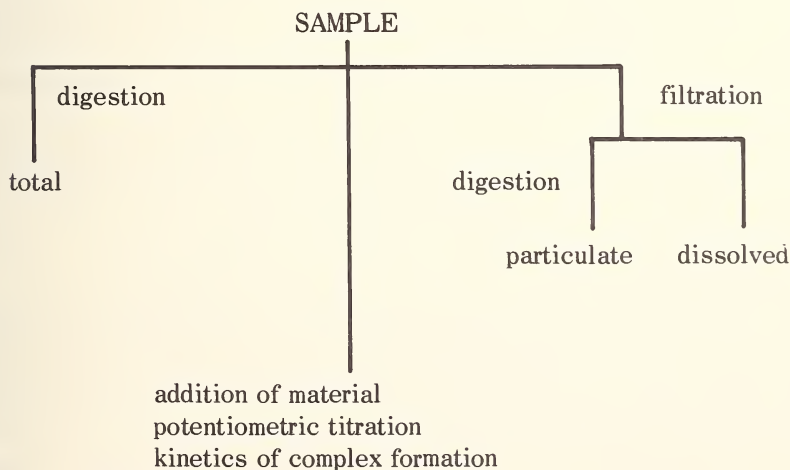


Figure 7. Analytical scheme for selective ion electrode determination of metals in aquatic environments.

## D. ANALYSIS FOR ORGANIC MATTER

### 1. Significance

Organic material in water may be naturally produced within the water body, such as plankton waste products, or derived from outside the water body, such as leaves detritus or by man-made waste products. Organic matter may be present in particulate, colloidal, or dissolved form, depending upon physicochemical characteristics of the water, *i.e.*, temperature, pressure, pH, ionic strength.

Organic constituents of certain industrial wastewaters are of general concern for one or more of a number of effects they may have upon receiving waters. The most readily apparent effect is that exerted directly on aesthetic quality by certain organic pollutants as a result of foaming, formation of slicks and films, offensive odors and tastes, discoloration, *etc.* Of equal significance are the effects of biologically oxidizable organic wastes on the depletion of dissolved oxygen levels in receiving waters, with concomitant disruption of the natural ecology of these waters. Other organic wastes may not degrade biochemically, but may rather persist and accumulate, thus constituting insidious long-term potential health hazards. The damaging effects of organic wastes on the quality of water for particular uses are summarized in Table 13.

The ability of organic matter of natural or pollution origin to complex with metals in aquatic environments is of utmost significance. For example, the complexation of ferric iron by certain organic compounds may prevent it from precipitating to the bottom and renders it more available to the aquatic biota. Organic complex formation with trace metals, *e.g.*, Cu, Pb, Cd, Zn, Co, Cr, *etc.*, may result in solubilizing these metals and in effect raises their concentration in the aqueous phase. The ability of aquatic biota to utilize organometallic complexes *vis-a-vis* free metal ions is under investigation and is considered to have significant ecological implications.

Several approaches are commonly used for characterization of organic matter in industrial waste effluents. These can be classified into two main categories. In the first category the damaging effect (pollutional effect) of the organic waste matter on the receiving water is estimated. This may be accomplished by diluting the waste effluent with the receiving water to a level corresponding to the dilution which will result in the receiving stream, and then characterizing appropriate pollution parameters, such as odor, color, carbon demand, chlorine demand, fresh-fish tainting, persistence (resistance to biodegradation) and treatability.



Table 13. Characteristics of organic pollution.

Water use	Damage to water quality for particular use
Domestic <sup>a</sup> or industrial process supply	<ul style="list-style-type: none"> <li>Taste and odor</li> <li>Carbon demand</li> <li>Chlorine demand</li> <li>Interference with coagulation</li> <li>Color</li> <li>Corrosion promotion</li> <li>Carbinogenic properties</li> <li>Toxicity to humans</li> </ul>
Production of fish	<ul style="list-style-type: none"> <li>Toxicity to fish or fish food</li> <li>Taint fish flesh</li> <li>Deoxygenation of water</li> <li>Promotion of filamentous organisms</li> <li>Sludge deposits</li> </ul>
Recreation	<ul style="list-style-type: none"> <li>Odor</li> <li>Color</li> <li>Floating matter</li> <li>Suspended matter</li> <li>Sludge deposits</li> </ul>
Agricultural irrigation	Toxicity to plants
Watering of livestock	Toxicity to animals

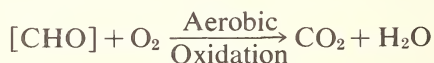
<sup>a</sup> This damage applies in cases where industrial waste is discharged to municipal sewers for combined treatment with domestic waste.

The second approach is based on both qualitative and quantitative analyses for the organic compounds. Analyses for organic compounds may involve either nonspecific or specific analytical methods. In the former case, analysis is done by measurement of oxidizable organic matter, such as the biochemical oxygen demand or the chemical oxygen demand, or by determining the total organic carbon or total organic nitrogen present in a given sample. Specific analysis, on the other hand, includes identification and quantitative determination of specific species present in the test solution.

## 2. *Nonspecific Analysis*

Nonspecific analytical methods are often based upon measurement of quantities of oxidizable organic material, either by biologically-mediated oxidation or by strictly chemical oxidation (by wet or dry combustion procedures). The procedure most commonly employed for measurement of the susceptibility of a waste to biological oxidation is the biochemical oxygen demand (BOD) test, while the chemical oxygen demand (COD) test is widely used for measurement of concentrations of organic matter oxidizable by a dichromate reflux method. Detailed descriptions of standard procedures employed for BOD and COD measurements are readily available in the literature [32,33].

**a. Biochemical Oxygen Demand.**—Basically the biochemical oxygen demand test is a bioassay procedure for determining the amount of biologically oxidized organic matter in terms of the amount of oxygen consumed. The test is done at controlled conditions. Measurements are based on the assumption that since the amount of oxygen required to convert a given quantity of biologically-oxidizable organic compounds to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  is fixed, it is possible to interpret BOD data semi-quantitatively in terms of gross concentrations of organic matter as well as oxygen consuming tendency. This is illustrated as follows:



where  $[\text{CHO}]$  refers to biodegradable organic compounds. Measurement is given in terms of the amount of oxygen consumed within five days "BOD<sub>5</sub>."

Similar to any bioassay technique the variables in the BOD test are (a) types, numbers and ages of microorganisms, (b) types and concentration of the organic substrates, (c) effects of temperature, mixing, *etc.*, (d) presence of antibiotic agents, (e) illumination which may promote photosynthetic oxygen production, and (f) presence of predator organisms.

One may certainly question—and many have—the use of such a simple expression for description of a complex biochemical process. The formulation of such a complicated reaction has been widely discussed, and numerous modifications have been proposed. It is beyond the scope of this presentation to cover this aspect of the BOD test, and the reader is referred to other sources for detailed discussions of this matter [82,83].

It is important to note that under most favorable conditions, the precision of the BOD test is in the order of  $\pm 20$  percent.

Because the dissolved oxygen content in streams, rivers, or lakes is of major concern from the standpoint of water pollution control, the BOD test has been extensively used for providing an estimation of the amount of oxygen likely to be consumed by a given amount of waste upon discharge to a receiving water. By utilizing a "seed" taken directly from the receiving water in question, actual conditions can be closely paralleled in that the measured consumption of oxygen will be that corresponding to the biological activity of the particular organisms indigenous to that receiving water.

Recent studies [84] have pointed out the advantage of determining the rate of biochemical oxygen demand, rather than the  $BOD_5$  *per se*. In an attempt to predict the effect of disposal of organic matter on the oxygen balance in the stream, the authors described a laboratory procedure to evaluate the assimilative capacity of receiving waters. The technique is based on the simultaneous determination of the rate of biochemical oxygen utilization and the rate of atmospheric oxygen uptake, using stream and waste waters, in appropriate rations.

**b. Chemical Oxygen Demand.**—The chemical oxygen demand test (COD) measures concentrations of organic materials which may be oxidized by a strong oxidizing agent. Depending upon the nature of the organic compounds present in water, the COD test may represent a fractional measure of total organic content.

A number of oxidants have been tested for possible use in the COD method, including: dichromate, permanganate, persulfate, ceric sulfate, perchloric acid, periodic acid, nitric acid, and numerous combinations of these and other reagents. Dichromate has been selected for the standard method on the basis of comparative testing with other reagents such as those listed above [33].

The COD test was originally intended to serve the combined functions of providing a more complete measure of the organic content of wastewaters than is afforded by the BOD test, while at the same time giving a rapid approximation to the ultimate oxygen requirements of the waste. Unfortunately, the test as presently described accomplishes neither of these objectives completely, in that oxidation of many organic compounds is not complete under the conditions of the test and, because certain biologically-oxidizable substances are not measured as COD while others, which are not available for biological oxidation are oxidized by the dichromate-sulfuric acid mixture.

### 3. *Specific Analysis*

**a. Elemental Analysis.**—A gross estimate of organic matter in water can be achieved by determining the total organic carbon (TOC) and in certain cases total organic nitrogen (TON) or total organic phosphorus (TOP).

The total carbon analysis test determines total soluble carbon within a matter of minutes and the method requires less than 0.5 ml of sample [85]. If particulate matter is excluded the test represents total soluble carbon and if inorganic carbon is excluded or corrected for, the results represent dissolved organic carbon.

The aqueous sample is injected directly into a combustion tube, heated to 970 °C in a constant flow of oxygen gas. Any organic matter is oxidized completely to CO<sub>2</sub> and water vapor on an asbestos packing impregnated with catalyst and these are carried from the combustion tube by the oxygen stream.

Dry combustion of the organic matter is usually done in the presence of a catalyst (*e.g.*, cupric oxide, cobaltic oxide, or asbestos-supported silver permanganate at elevated temperatures of 900 to 1000 °C).

Organic nitrogen determinations are commonly done by the Kjeldahl method. This method is applicable to many types of organic compounds although it is sometimes referred to as a method for aminoid or albuminoid nitrogen.

The procedure is based on destroying organic material with sulfuric acid in the presence of various catalysts, and the nitrogen is converted to ammonium acid sulfate. The ammonia is liberated and either titrated or determined colorimetrically [32,33]. One of the principal problems of this method is that certain organic-bound nitrogen structures cannot be easily transformed to ammonia [86]. Mercury is considered an adequate catalyst for the digestion of this type of compounds.

Several methods utilizing various combustion techniques separately or in conjunction with the Kjeldahl procedure have been proposed. The reader is referred to the recent review articles [86,87].

For determination of organic phosphorus, organic material is first broken down by wet combustion with mixtures of sulfuric and nitric acids. The phosphoric acid produced then can be determined either gravimetrically as ammonium phosphomolybdate or colorimetrically [32,33].

**b. Constituent Analysis.**—Specific analysis of the organic constituents in natural and wastewaters is not an easy task. Direct application of conventional analytical techniques is frequently not possible because the quantities present may be well below detectable limits. Additionally, the



presence of interferences may create difficulties for the application of certain separation techniques. One of the major problems associated with the isolation and separation of organic compounds from water samples is the biochemical degradation or chemical transformation of these compounds resulting from bacterial action, temperature effects, surface catalysis, solvent-solute and solute-solute interactions, *etc.*

It is frequently necessary to concentrate sample solutions to bring them within the detectable limits of certain analytical procedures. Concentration by removal of water can be achieved by either evaporation or freezing. For evaporation, vacuum distillation at low temperatures is generally preferred. Partial distillation and steam distillation techniques have also been employed [88], and freeze concentration techniques have been used very effectively [89]. One of the main advantages of freeze concentration is that the materials to be concentrated are kept at low temperature during the entire process; thus the chances for loss of volatile constituents and/or alteration of the nature of organic substances of interest are minimized.

Reverse osmosis with cellulose acetate membranes can be used effectively to concentrate both low and high molecular weight organic compounds [90]. In this technique, pressure greater than the osmotic pressure is applied to the sample solution, resulting in the flow of water through the membrane, with a high degree of retention of the organic solute. The advantage of the membrane-osmotic method for concentrating dilute solutions of organics is that it is an *in situ* separation technique which does not involve changes in phase or changes in temperature.

Carrying the same concept further, all the water can be removed from the sample by evaporating till dryness or freeze-drying. These techniques, however, are used primarily to get a rather rough estimate of the non-volatile organic content.

**c. Separation Techniques.**—A variety of separation techniques may be used, singularly or in combination, for isolation and concentration of organic matter in natural and waste waters. Among these are distillation [88], solvent extraction [91], precipitation and crystallization [92], adsorption [93], gas chromatography [94], paper chromatography [95], and thin layer chromatography [96].

Partial distillation, in which the sample flows through the distillation cell continuously and only a small portion is distilled (10 percent), has been applied to oil refinery waste effluents [88].

Adsorption chromatography is one of the best methods available for rapidly concentrating or extracting solutes from dilute aqueous solution. Compared with the volume of the solution used, a relatively small volume

of absorbent is required for the separation. The solute normally can be recovered from the absorbent in a small volume eluting agent.

Adsorption chromatography with activated carbon has been used for more than a decade for separating organic compounds from surface waters and certain industrial effluents [93,96]. Much of the work leading to the development of this procedure was done at the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, in the early 1950's [96]. For this procedure, a carbon column, often referred to as the "carbon filter" is used in conjunction with a sand prefilter and/or a presettling tank. The efficiency of the method is a function of the rate of flow of the sample solution, particle size of the absorbent, type and characteristics of the adsorbate(s), and physicochemical properties of the sample solution such as temperature, pH, ionic strength and turbidity.

The organic matter collected on the adsorption column is recovered by solvent extraction using chloroform and ethyl alcohol. These two extracts are further fractionated by means of various solvent extraction schemes as shown in Figures 8 and 9.

The carbon filter technique, as it is used now [32,33,98] does not quantitatively separate the total organic content of a water or waste solution. Recoveries may range from approximately 50 to 90 percent, and replicate samples may agree within only about  $\pm 10$  percent. In spite of these drawbacks, the carbon filter technique is useful for qualitative collecting of organic matter from dilute waste effluents. It can be used to advantage for screening purposes, as well as for monitoring industrial waste effluents.

Gas chromatography has proven to be a useful tool for analysis of natural waters and wastewaters, being used in the majority of cases to separate and identify components of extracts from carbon filters or from other separation processes [91-94]. In a few cases, however, water samples have been directly injected for analysis of organic content [94,100,101].

Paper chromatography and thin-layer chromatography also find application for separation and identification of organic constituents in wastewaters. Phenols, cresols, xyleneol, and other industrial and biological phenols have been determined in the microgram per liter concentrations in polluted waters by paper chromatography [102]. Chlorinated hydrocarbon pesticides have been determined at concentrations of two micrograms per liter or less in carbon-chloroform extracts [103].

Thin-layer chromatography has been used effectively for removing interfering substances from pesticides prior to gas chromatographic analysis [96]. Christman has discussed the advantages of thin-layer chromatography over paper chromatography for separation and identification of organic chromagenic agents in natural waters [89]. Similarly the separation

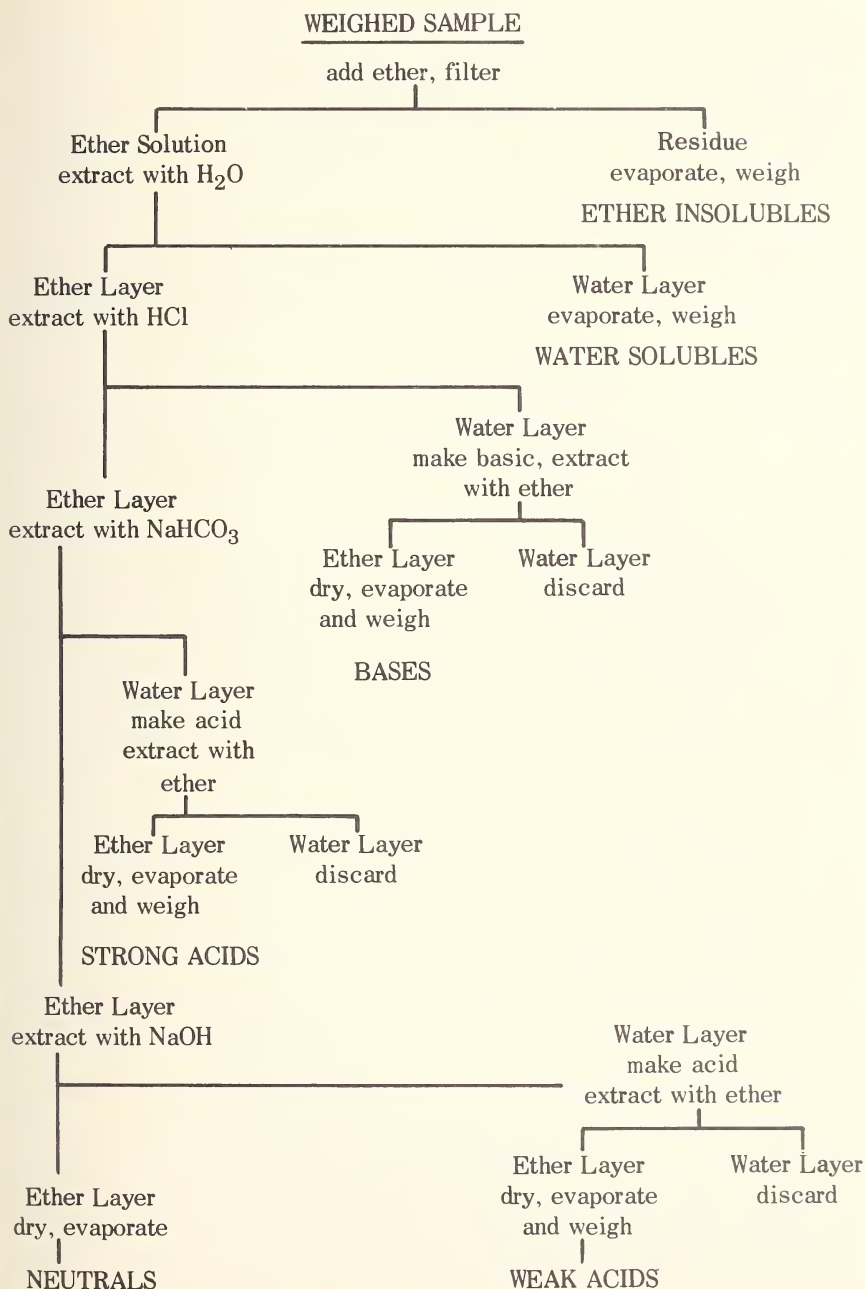


Figure 8. Liquid extraction scheme for organic compounds.

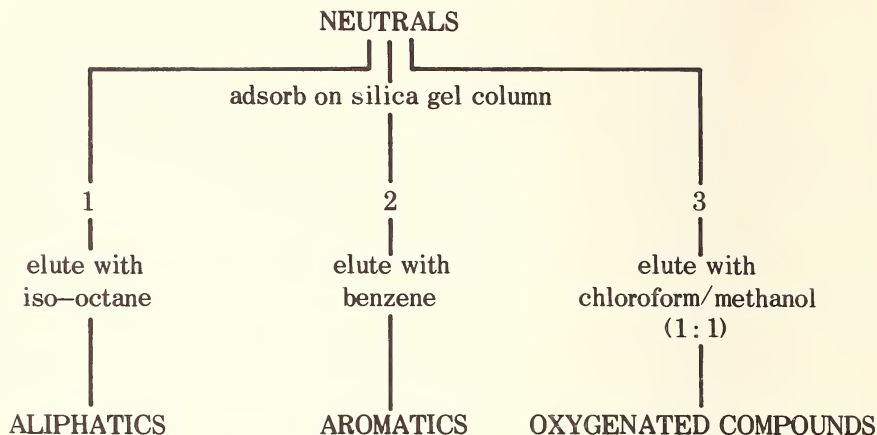


Figure 9. Chromatographic separation of neutrals.

of various herbicides can be effectively done by thin-layer chromatographic techniques [96].

**d. Identification Techniques.**—Spectrometric techniques can be used for the identification of organic compounds in natural and wastewaters, *e.g.*, infrared, ultraviolet, mass spectroscopy and nuclear magnetic resonance techniques. For analysis of natural and wastewaters, analytical spectrometry must generally be preceded by separation of the organic material from the wastewater sample by one or more of the techniques discussed previously.

Near-infrared spectrometry offers some advantages when interferences occur in the "fingerprint" region from 7  $\mu\text{m}$  to 12  $\mu\text{m}$ . Measurement in the near-infrared region (1  $\mu\text{m}$  to 3  $\mu\text{m}$ ) are only useful for detecting and subsequently identifying functional groups which contain unique hydrogen atoms, and are not characteristic of the organic molecule as a whole.

A modification of infrared spectrometry which offers unique possibilities for analysis of organic matter in water involves attenuated total reflectance [104,105]. This method is based on the passage of a monochromatic or monochromatically scanned light into a crystal of suitable material, in contact with the test solution, and detection of the transmitted light intensity. Changes in light intensity are related to changes in the type and concentration of "light active" substances in contact with the crystalline material. The principle is based on energy reflection at the interface between media of different refractive indices [106].

UV-spectrometry finds wide application in industrial processes for monitoring the composition of reaction mixtures. Monitoring of trace or-



ganic material in industrial effluents by UV-spectrometry has been reported [107]. The method is based on measuring UV absorption spectra for compounds having aromatic or conjugated unsaturated molecular configurations. The instrument employed for these analyses used a mercury discharge lamp with a principal UV-radiation at 2537 Å. The author has reported a sensitivity of 10 mg/l of phenol in water.

Mass spectrometry has not as yet been widely used for analysis of wastewaters. Melpolder [108] has described the distillation of volatile organic compounds from oil refinery wastewater and subsequent identification by mass spectrometry. Because of the high training level required for operating personnel, the data handling problems, and the cost of instrumentation, this technique has been restricted primarily to research applications, and then to only a few major water pollution control laboratories. The technique does, however, offer considerable possibilities; for example, mass spectrometry has been used to detect phenols in nanogram quantities in the effluent from a gas chromatographic column [109].

Fluorescence spectrophotometry has been applied successfully for analysis of lignin sulfonates in the waste effluents from Kraft sulfite pulping operations and for analysis of organic color-forming compounds in natural waters [110]. Traces of carbohydrate in the nanogram per liter range have been determined in water samples using spectrofluorometry [111]. The sensitivity of the method and the effects of environmental factors have been discussed [111].

#### 4. *Biodegradability of Organic Compounds*

Biodegradability tests are basically designed to estimate the extent to which organic compounds may be oxidized biochemically and an industry must be concerned with the question of whether its organic products or waste materials can be degraded or assimilated efficiently by existing biological waste treatment processes. Biodegradability may be considered as a measure of the susceptibility of organic material to microbial metabolism. This property is not well defined and there is no single standard test for its measurement. It constitutes, however, a dominant mechanism for the removal of organic pollutants from water, both in self-purification processes in natural waters and in the accelerated biological processes of waste treatment. Biodegradation can occur aerobically or anaerobically, depending on the availability of atmospheric oxygen. The process is affected by a variety of environ-

mental conditions. As discussed previously, organic compounds are not equally susceptible to biodegradation; some are readily metabolized, others are more resistant (refractory compounds). Principal factors involved in any biodegradation process are as follows: (a) type and number of microorganisms; a mixed culture of organisms, as in sanitary sewage, possesses a remarkable capacity to adapt to strange or different organic materials while single cultures may not be effective, (b) structure and concentration of organic materials; certain organic compounds, *e.g.*, certain pesticides and surfactants, are relatively resistant to biodegradation in comparison to simple carbohydrates. The concentration of the organic material is also significant. High concentrations of "sugars" in certain wastewaters may inhibit biodegradation, yet upon dilution in a receiving water the sugars will be easily degraded, resulting in a water pollution problem due to oxygen depletion, (c) environmental factors; factors such as temperature mixing, viscosity, *etc.* of the wastewater are quite significant in dictating the extent and rate of biodegradation and its effect on the ecology of the receiving environment (air, water, or soil).

Much of the work which has been carried out on biodegradation has been concerned with surfactants, and in particular with the "hard" branched-type ABS and the "soft" straight chain LAS [112,113].

A number of techniques have been used to test for biodegradability, such as the Warburg respiration technique [114], activated sludge tests [115], shake-flask experiments [115], and river die-away measurements [116]. A comprehensive critique of various biodegradability tests has been reported [117]. Sewage microorganisms are commonly used for biodegradability measurements because of availability, and to avoid the need for maintaining standard cultures [117]. Biological oxidation has been followed by measurement of one or more of the following parameters: (a) the rate of disappearance of the organic compound under test, (b) the rate of appearance of biodegradation by-products, (c) growth rate of microorganisms, and (d) dissolved oxygen consumption.

## E. ANALYSIS FOR INORGANIC ANIONS

### 1. Significance

Inorganic anions of significance in water pollution characterization may be categorized into (a) halides, *e.g.*,  $F^-$ ,  $Cl^-$ ,  $Br^-$  and  $I^-$ , (b) nutrient anions, *e.g.*,  $PO_4^{3-}$ ,  $NO_3^-$  and  $NO_2^-$ , (c) sulfur anions and (d) cyanides

and thiocyanate. The significance of each group, insofar as water quality is concerned, varies widely and generalities cannot be drawn. Of particular interest to the public nowadays is the problem of disposal of phosphates in natural waters. It is claimed that increasing the phosphorus level will result in the acceleration of the rate of eutrophication of receiving surface waters. This is considered a serious problem since current sewage treatment processes do not remove phosphorus from sewage effluents.

## *2. Separation and Concentration Techniques*

Separation and concentration techniques for metal ions are generally applicable also for nonmetal inorganic species. Evaporation, precipitation, ion exchange, solvent extraction, and partial freezing are frequently used ion exchange being particularly well suited for anion separations. Anion exchange chromatography has been used rather extensively for separations of species found in waters and wastewaters [117].

## *3. Measurement Procedures*

Phosphorus may be present in industrial waste effluents either as inorganic phosphates (ortho-, meta-, or poly-phosphates) or in organic combination. The most common analytical method for inorganic phosphorus is based on the colorimetric determination of the phosphomolybdenum blue complex [32,33]. The test is sensitive to orthophosphates and not condensed phosphates. Polyphosphates and metaphosphates are then estimated as the difference between total phosphates (hydrolyzed samples) and orthophosphates (nonhydrolyzed samples).

Orthophosphates react with ammonium molybdate in acid medium to form the phosphomolybdic acid complex, which when reduced yields the molybdenum blue color which may be determined colorimetrically. The sensitivity of the test is largely dependent on the method of extraction and reduction of the phosphomolybdic acid. Aminonaphthol-sulfonic acid [33], stannous chloride, (Deniges Method) [118], metal sulfites (Tschopp reagent) [119], and ascorbic acid [120] have been used in the reduction step. The stannous chloride method is considered most sensitive and best suited for lower ranges of phosphate concentration.



A number of substances have been reported to interfere with the phosphate determination [33]. Arsenic, germanium, sulfides, and soluble iron above 0.1 mg cause direct interferences. Tannins, lignins and hexavalent chromium will cause errors only for analysis of phosphate concentrations below mg/l.

Indirect UV spectrophotometry and atomic absorption methods have been developed for phosphates and silicates [121]. These techniques are based on the selective extraction of molybdophosphoric acid and molybdosilicic acid, followed by ultraviolet molecular absorption spectrophotometry and/or atomic absorption spectrophotometry. The molybdophosphoric and molybdosilicic acids are formed in acidic solution by addition of excess molybdate reagent. Molybdophosphoric acid is extracted with diethyl ether from an aqueous solution which is approximately 1 *M* in hydrochloric acid. After adjusting the hydrochloric acid concentration of the aqueous phase to approximately 2 *M*, the molybdosilicic acid is extracted with 5:1 diethyl ether-pentanol solution. The extracts of molybdophosphoric and molybdosilicic acids are subjected to acidic washings to remove excess molybdate. Each extract is then contacted with a basic buffer solution to strip the heteropoly acid from the organic phase. The molybdate resulting from the decomposition of the heteropoly acid in the basic solution is then determined either by measurement of the absorbance at 230 nm using ultraviolet spectrophotometry or by measurement of absorbance at the 313.3 nm resonance line of molybdenum by atomic absorption spectrophotometry. The optimum concentration ranges are approximately 0.1 - 0.4 mg/l of phosphorus or silicon for indirect ultraviolet spectrophotometry and 0.4 - 1.2 mg/l for indirect atomic absorption spectrophotometry.

#### *4. Potentiometric Membrane Electrodes*

The recently developed "selective ion electrodes" offer unique analytical capabilities for the determination of  $F^-$ ,  $Cl^-$ ,  $Br^-$ ,  $I^-$ , and  $CN^-$ ,  $SCN^-$ ,  $NO_3^-$ , and  $S^{2-}$ . The principle and performance characteristics of these electrode systems were discussed earlier.

The fluoride ion membrane electrode finds its widest application in the analysis of fluoridated water supplies [80]. Free fluoride ions are determined in water samples buffered to pH 5.2 by an acetate buffer. Total fluorides are determined after the addition of a masking agent, *e.g.*, citrate, which forms stable, nonvolatile complexes with aluminum and



measuring fluorides at pH 5.2. The addition of a masking agent is necessary to release the bound fluorides, *e.g.*,  $(\text{AlF}_6)^{3-}$ . In this procedure the fluoride ion-potentiometric membrane electrode is used to provide quantitative information on free and complexed fluorides. This should be highly significant in assessing the effect of free *vis-a-vis* complex fluorides on the prevention and control of dental caries.

Similarly, the sulfide ion membrane electrode can be used to characterize free sulfides in natural and waste waters [21]. Potentiometric measurements of  $\text{S}^{2-}$  can be related to the concentrations of  $\text{H}_2\text{S}$ ,  $\text{HS}^-$ , and  $\text{S}_\text{T}$  (analytical concentration of free sulfides).

Cyanide, nitrate and chloride ions, in natural and wastewaters, may be also determined by selective ion electrodes [80]. These applications are subject to the same general limitations on sensitivity and selectivity as those for previously mentioned membrane electrodes. It is important, however, to establish the electrode performance characteristics in any given water prior to its application. For example, the nitrate electrode is quite accurate and precise (1 - 5 percent error) in pure nitrate solutions, and the lower limit of sensitivity is about 0.6 ppm  $\text{NO}_3^-$  which is sufficient to give meaningful electrode response at any nitrate level in surface and underground waters in the USA. Nevertheless, the electrode's liquid ion-exchanger deteriorates sufficiently to cause significant errors after three to four weeks of operation, after which the electrode must be rejuvenated and recalibrated.

One of the main problems with the nitrate electrode is the effect of interferences by  $\text{Cl}^-$  and  $\text{HCO}_3^-$ , commonly found in all types of waters.

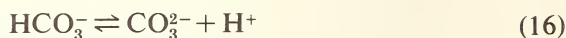
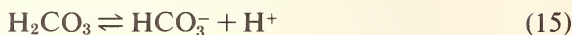
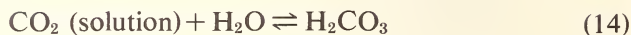
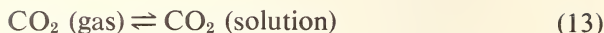
## F. ANALYSIS FOR DISSOLVED GASES

### 1. Significance

The gases of significance in water pollution characterization are primarily those associated with biological activities in the aquatic environment *e.g.*,  $\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{H}_2\text{S}$ ,  $\text{NH}_3$ ,  $\text{CH}_4$ , *etc.* Carbon dioxide and oxygen are particularly of significance in assessing biological productivity. Hydrogen sulfide, methane and gaseous ammonia, if present in natural and wastewaters, are considered indicators of anaerobic conditions.

The analysis for  $\text{CO}_2$ ,  $\text{H}_2\text{S}$ , and  $\text{NH}_3$  is frequently based on acid-base equilibrium calculations. For example in the case of  $\text{CO}_2$  the following

reactions occur:



In spite that the hydration of dissolved carbon dioxide and the dehydration of carbonic acid are relatively slow, it is possible to derive equilibrium expressions for  $\text{CO}_2$  concentrations [123]. By combining the expressions for carbonate alkalinity,  $[\text{Alk}]$ , the solubility of carbon dioxide and carbon dioxide ionization constants ( $K_1$  and  $K_2$ ), it is possible to derive the following expression:

$$[\text{CO}_2] = [\text{Alk}] \frac{a_{\text{H}}}{K_1 \frac{\alpha_0}{\alpha_s} \left[ 1 + \frac{2K_2}{a_{\text{H}}} \right] a_{\text{H}_2\text{O}}} \quad (17)$$

where

$$[\text{Alk}] = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] - [\text{H}^+], \quad (18)$$

$$K_1 = \frac{a_{\text{H}}[\text{HCO}_3^-]}{a_{\text{H}_2\text{O}}a_{\text{CO}_2}}, \text{ and } K_2 = \frac{a_{\text{H}}[\text{CO}_3^{2-}]}{[\text{HCO}_3^-]} \quad (19)$$

and  $\alpha_0$  and  $\alpha_s$  are the solubility coefficients of carbon dioxide in pure water and sample, respectively.

## 2. Separation and Concentration Techniques

Dissolved gases in natural and waste waters may be readily separated by vacuum degasification or by one or more of various stripping techniques. Stripping is essentially a gas-liquid extraction procedure in which an inert carrier gas is bubbled through a sample to carry off the dissolved gases for further separation, concentration, or detection. Gas transfer efficiency in such systems is primarily dependent on the gas-liquid interfacial area and on the degree of mixing.

Gas-exchange separation can be carried out as either a batch or a continuous flow process. In one design, a continuous mixed stream of sample and carrier gas (nitrogen or hydrogen) is forced through an aspirator nozzle under fifty pounds of pressure [124]. In another design, the dissolved gases are stripped from the test solution by means of multiple spinning discs rotating at high speed [125,126]. Detection of the stripped gases in the stream of carrier gas may be done by measurement of paramagnetic susceptibility, thermal conductance, *etc.* [124].

The gas stripped from a wastewater sample may be separated into its various components by gas chromatography [126]. Several modifications of this technique have been reported [124-128]. By choosing appropriate detectors, it is usually possible to analyze simultaneously for almost all gases of interest in a water or wastewater sample.

### 3. Sampling

*In situ* analysis is probably the most effective way to analyze for dissolved gases *e.g.*, dissolved oxygen. Certain precautions should be taken in cases where water samples are collected and stored for subsequent analysis. The sample must not remain in contact with air nor be agitated; either condition will cause a change in dissolved gas levels. Samples from any depth or from waters under pressure require special procedures to eliminate the effects of changes in pressure and temperature on sampling and storage. Detailed description of procedures and equipment for proper sampling of waters under pressure as well as waters at atmospheric pressure are available in the literature [34].

### 4. Measurement Procedures

For illustrative purposes, the analysis for dissolved oxygen is briefly discussed in this presentation. A detailed discussion on this subject can be found elsewhere [129].

The oldest and one of the most popular methods for the analysis of dissolved oxygen is the Winkler Test [130]. Originally reported about 75 years ago, the Winkler procedure is still the basis for the majority of titrimetric procedures for dissolved oxygen. The test is based on the quantitative oxidation of manganese (II) to manganese (IV) under alkaline conditions. This is followed by the oxidation of iodide by the manganese

(IV) under acidic conditions. The iodine so released is then titrated with thiosulfate in the presence of a starch indicator.

In applying the Winkler Test for oxygen determinations in natural and wastewaters, full consideration must be made of the interfering effects of oxidizing or reducing materials in the sample. The presence of certain oxidizing agents liberates iodine from iodide (positive interference), and the presence of certain reducing agents reduces iodine to iodide (negative interference). Reducing compounds may also inhibit the oxidation of the manganous ion. Certain organic compounds have been found to interfere with the Winkler Test in a different way. Surface-active agents for example, have been reported to hinder the settling of manganic oxide floc, thus partially obscuring the end point of the final titration with thiosulfate [129].

Several modifications of the Winkler Test have been devised to minimize the effect of interferences found in different waters [129].

The precision and accuracy of titrimetric procedures for dissolved oxygen may be considerably improved by using better end-point detection techniques. Potentiometric detection of the iodometric end-point improves sensitivity to about  $\pm 0.001$  mg/l of iodine [129]. "Dead stop" end-point detection (an amperometric technique) offers an extremely sensitive as well as accurate measurement [129]. The procedure is quite simple and utilizes two smooth platinum electrodes with a small potential difference (from 15 mV to 400 mV, depending on the sensitivity required). Diffusion current is measured during the course of the titration. No attempt is made to control the potential of either electrode; only the potential difference is controlled. The end-point is indicated by discontinuation of current flow in the cell. As long as free iodine remains in the solution, the chief electrode reaction under the influence of the applied voltage is the oxidation of iodide to iodine at the anode and the reverse process at the cathode. At the end-point when all free iodine has been removed, the iodine to iodide reaction can no longer occur and the cell current comes to a "dead stop." Since the thiosulfate/tetrathionate reaction is highly irreversible and proceeds at only a minute rate under the influence of the applied voltage, no detectable current is observed at and beyond the end-point. Ordinarily, the end-point is so easily detected that there is no need for a graphical estimation of its position. By using sensitive current-measuring devices the end-point can be established to an accuracy of  $\pm 0.01$  microgram iodine in a 100-milliliter sample, or 1 part in 10 billion.

Coulometric titration of dissolved oxygen by *in situ* electrochemical generation of iodine has been used with considerable success [131]. The procedure consists of the successive additions of standard solutions of



MnSO<sub>4</sub>, KOH + KI, H<sub>2</sub>SO<sub>4</sub>, and an excess of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to the test solution. The iodine formed electrolytically reacts with the residual thiosulfate in solution. The electrolytic current is held constant by varying the potential, and the equivalence point is conveniently detected by the dead-stop end-point method.

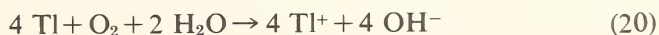
The procedure is very accurate, to within 0.02 μg/l. It has a distinct advantage in that, because the titrant is generated in solution, errors caused in conventional titrations by contact with air are eliminated.

Direct colorimetric methods of analysis for dissolved oxygen are based on the interaction of molecular oxygen with an oxidation-reduction indicator to give a color change. One of the most commonly used indicators for the detection of oxygen in solution is methylene blue; others are indigo carmine and safranin-T.

In the presence of dissolved oxygen a reduced methylene blue solution exhibits a blue color, and in the absence of dissolved oxygen it is colorless. This indicator has been used in the relative stability test for sewage effluents [33]. Quantitative colorimetric determinations of dissolved oxygen can be made with indigo carmine dyes. Indigo carmine in the reduced state reacts with oxygen to give a color change through orange, red, purple, blue, and finally a blue-green in the completely oxidized form. Colorimetric procedures are subject in general to a variety of interferences which limit their applicability to industrial wastewaters.

A radiometric procedure for monitoring dissolved oxygen is based on the quantitative oxidation of radioactive thallium-204 by oxygen in the test solution [132]. Thallium-204 is primarily a beta emitter with a half-life of 3.6 years; therefore, decay over several months does not greatly reduce the sensitivity of the technique. The apparatus consists of a column of radioactive thallium electrodeposited on copper turnings, and two flow-type Geiger-Mueller counters.

The technique involves passing the test solution by one of the Geiger-Mueller counters to detect background beta-activity, then through the column where the following reaction occurs:



The radioactive thallium in the effluent from the column is detected by the second Geiger-Mueller counter. One milligram of oxygen liberates  $25.6 \times 10^{-3}$  g of <sup>204</sup>Tl. The counting rate is directly proportional to the oxygen concentration in the test solution.

The sensitivity of the test using a column with a specific activity of 2.04 millicurie per gram of thallium is about 0.2 mg/l. That is to say, a test solu-

tion containing 0.2 mg/l produces a  $^{204}\text{Tl}$  counting rate equal to the background counting rate of the detector. As a rule, because of the randomness of radioactive disintegrations, the precision of this method is  $\pm 2$  percent. It is important to note that oxidizing agents and changes in the pH of the test solution may interfere with the test.

A few coulometric methods of analysis for dissolved oxygen have been reported. For purposes of orientation, it is helpful to note that air-saturated water at 25 °C and under 750 mm Hg air pressure contains 8.18 mg of dissolved oxygen per liter. This in terms of coulometric response is 0.1083 A·s/g, a rather large quantity. Two coulometric procedures are of interest [129,133]. In one method chromous ions are generated electrolytically and are then reoxidized by the oxygen in solution. Detection of chromous ions marks the end of the titration. Another coulometric method is based on the interaction between oxygen and an ammonia-copper complex  $\text{Cu}(\text{NH}_3)_2$ . This is followed by the reduction of the oxidized ammonia-copper complex on a platinum cathode.

Use of a constant-potential derivative coulometric system was reported recently [134]. The working electrode was composed of tiny metal spheres packed in a ceramic tube. No field experience has been reported with this system, however.

Voltammetric analyses for dissolved oxygen have been carried out with various degrees of success using rotating platinum electrodes and dropping mercury electrodes. The main difficulty in using such electrode systems in natural and wastewaters is the presence of surface active and electroactive interferences which frequently cause "electrode poisoning." A detailed discussion of the effects of surface active agents on the polarographic oxygen determination is available in the literature [135].

Various modifications of the dropping mercury electrode system have been developed for continuous monitoring of dissolved oxygen [125,135]. In the absence of interferences, the sensitivity of this technique ranges from 0.05 to 0.10 mg of dissolved oxygen per liter.

Oxygen-sensitive galvanic cells have been used for some time for analyses of water effluents [135]. These are made of galvanic couples of an inert metal cathodes (*e.g.*, lead, zinc or antimony) [136]. The cathodic reduction of molecule oxygen results in a galvanic current proportional to the concentration of dissolved oxygen in the test solution. Change in the pH and the conductivity of the test solution influences the oxygen measurement.

The usefulness of this type of cell is limited because the electrode system may be easily poisoned. The electrode is in direct contact with the test solution and surface-active compounds as well as other suspended

material frequently adsorb on its surface, particularly in wastewaters. To prevent incrustation, a small amount of HCl may be added to the water by a dosing device ahead of the galvanic cell [135].

At the present time it appears that voltammetric membrane electrodes offer one of the most useful techniques for analysis of dissolved oxygen in wastewaters. The unique feature of such electrode systems is that of the membrane separating the electrode from the test solution. Two main types are presently available, the voltammetric type [136] and the galvanic cell type [137]. The two types are similar to operating characteristics; in the voltammetric type an appropriate e.m.f. source is needed, however, while the galvanic type is basically an oxygen energized cell.

Oxygen membrane electrodes have three main components, the membrane, the oxygen-sensing element, and the electrolyte solution. The membrane, the unique feature in such electrode systems, serves in three different capacities. First, the membrane acts as a protective diffusion barrier separating the sensing element from the test solution. Since plastic membranes are permeable to gases only, oxygen molecules pass through, but electroactive and surface-active contaminants present do not. The possibility of poisoning the sensing element is thus minimized.

Second, the membrane serves to hold the supporting electrolyte in contact with the electrode system and thus makes it possible to determine oxygen in gaseous samples as well as in nonaqueous solutions such as industrial wastes [138].

The third advantage, discussed previously, is that the membrane constitutes a finite diffusion layer, the thickness of which is independent of the hydrodynamic properties of the test solution.

A detailed discussion of the principle, operating characteristics, applicability, and limitations in the use of oxygen membrane electrodes may be found elsewhere [139,140].

Polymeric membranes used with oxygen membrane electrodes show selective permeability to various gases and vapors. Gases reduced at the potential of the sensing electrode (*e.g.*, SO<sub>2</sub> and halogens) cause erroneous readings, but these gases rarely exist in a free state in aqueous systems. Other gases capable of permeating plastic membranes may contaminate the sensing electrode or react with the supporting electrolyte, *e.g.*, CO<sub>2</sub> and H<sub>2</sub>S.

Oxygen membrane electrode systems have been used extensively in laboratory and field analysis as well as for continuous monitoring purposes. Some of the main operational problems associated with the use of these electrode systems have been the effect of mixing in the test solution on the electrode sensitivity and short term stability which necessitates

frequent calibration. In monitoring operations, the accumulation of inert or biological material on the membrane surface has caused a lot of nuisance. In addition, it has been practically impossible to reproducibly change and mount the membranes without a change in the membrane thickness and permeability characteristics.

These problems can be minimized by the use of a "three electrode" voltammetric membrane electrode system and pulse voltammetry instead of steady state measurements [141-143]. Using an optimum pulse time and frequency, the new electrode system exhibits 100 times higher sensitivity and a remarkable long term stability. One of the main advantages of the pulse voltammetric technique is that measurement is independent of the membrane permeability and thickness. This is realized by comparing the steady state and transient diffusion current equations,

$$i_{\infty} = zFAP_m \frac{1}{b} a_{O_2} \quad (21)$$

$$i_p = zFA \left( \frac{D}{\pi t} \right)^{1/2} a_{O_2} \quad (22)$$

Equation 22 indicates that the peak current  $i_p$  is independent of the membrane permeability coefficient " $P_m$ " and thickness " $b$ ." Under these conditions deposition of material on the membrane and changes in the mixing characteristics of the test solution will have no effect on dissolved oxygen measurement.

## G. LEGAL AND ADMINISTRATIVE IMPLICATIONS

### 1. Enforcement Procedures

Enforcement of water pollution control programs is primarily based on water quality analyses. Too frequently, the analytical chemist is asked to provide data on which certain legal actions have to be made. Under these conditions the analytical chemist may have to provide evidence on the authenticity of the sample and the reliability of the measurement.

Enforcement procedures and its legal basis are beyond the scope of this article. However, it may be useful to illustrate the demands on analysis by taking for an example the federal enforcement procedures.



Water quality standards for interstate streams and their classifications are being adopted. The first responsibility for enforcement lies within the state and the final responsibility is that of the federal government.

In the case of a violation, a notice is issued to the violator and to the state for taking corrective actions within 180 days. Enforcement in court is primarily based on providing evidence of violation and to prove that the violation endangers human health and welfare. Meanwhile, it may be necessary to prove that compliance to the water quality standards is technologically and economically feasible.

Another enforcement mechanism available to the federal government is that of the "Federal Conference Procedure." If pollution from one state is causing harm to human health and welfare in another state or causing economic damages to interstate fish or shellfish marketing, a federal conference is held and the states are invited to participate. The conference does not constitute judiciary proceedings. A federal report is presented and the states are asked to execute corrective measures within six months.

Usually federal conference procedures result in extended programs of further fact gathering. Only after several other steps the case may be finally submitted to the Attorney General for court action.

More recently, the "Oil Pollution Act" gives enforcement responsibilities to the Coast Guard and the Environmental Protection Agency. Enforcement requires the proof that pollution is caused by oil, a petroleum product or traces of oils in products. Penalties for discharge and cost of cleanup are decided in court.

Pollution of surface waters by discharges from vessels, *e.g.*, pleasure boats, barges, cargo ships, *etc.*, falls under the "Oil Pollution Act." The Environmental Protection Agency is preparing standards for effluent discharges from such vessels. Enforcement will be the responsibility of the Coast Guard.

The Rivers and Harbors Act of 1899 (Refuse Act), which has been inactive for several decades, was recently applied to water pollution control. This is based on requiring industry to apply for permits for discharge of effluents into navigable waters. Applications for permits should be accompanied by information on the waste composition. It is the applicant's responsibility to supply these data.

The U.S. Corps of Engineers is the agency authorized to issue these permits. Evidence of violation is provided by the Environmental Protection Agency and enforcement is under the jurisdiction of the Justice Department. Violators may be brought to court under criminal proceedings

in cases of nonpermitted discharge or under civil proceedings (injunction) where irreparable damage of the receiving water is proven.

## *2. Problems of Collecting Evidence*

One of the main problems facing water analysts is how to collect evidence in case of violation of water quality regulations. This may involve the collecting of "valid" samples and performing certain analyses which may be challenged in court.

Sampling and analytical procedures, commonly used by regulatory agencies and industry, are listed in the "Standard Methods of Analysis for Water and Wastewater" [33]. The validity of these methods of analysis is generally accepted for purposes of legal proceedings. If the analyst chooses to use different methods of analysis, he has to provide the evidence that they are at least equivalent and are not less reliable than the standard methods.

One look at the latest Standard Methods [42] will give the impression that these procedures are generally out of date and in certain cases they lack the required sensitivity and accuracy. This is particularly true in the case of trace metals or trace organic analyses.

Another point of concern is related to the availability of adequate analytical capabilities to certain industries and government in order to enforce water quality standards and pollution control regulation. Presently, there is a scarcity of qualified analytical chemists, with appropriate backgrounds who are interested in water quality characterization on a day-to-day basis. In most cases, the responsibility of water quality analysis has been left to technicians or plant operators.

The seeming lack of opportunity or the nonattractiveness of the water pollution control field to analytical chemists should be blamed, in part, on the managers and program directors. There is an apparent apathy, especially in government agencies, to give the analytical chemist more responsibilities on the managerial level. This stems from a belief that chemical analysis *per se* is a supporting function to the main effort and responsible decision-making positions should be filled by engineers, lawyers or physicians. This is reflected in salary and promotional opportunities which are in contrast to the recognition earned by analytical chemists in other governmental and industrial agencies.

More exasperating is the fact that little emphasis is given in our educational institutions to the analytical chemistry of environmental quality.

Formal teaching in analytical chemistry is exclusively technique oriented with little or no emphasis on applications. A majority of educators believe that if the student is taught the fundamentals, he is capable to deduce the applications himself. This may be true, but it is indeed a very slow, expensive process and for a nonexperienced student, it may take him years to "deduce" these applications on his own. If we believe in the merit of specialization, then it is appropriate to offer problem oriented analytical chemistry courses dealing with health and pollution control.

On the national level, the National Bureau of Standards should participate and take a leading role in formulating and adopting standard methods for the Examination of Water and Wastewater. This could be in the form of analytical reference services for the purpose of establishing the applicability and limitations of classic and advanced water pollution measurement procedures.

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## PANEL DISCUSSION

### Analytical Problems in Water Pollution Control



#### CHAIRMAN

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#### PANEL

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Vincent P. Guinn, Professor of Chemistry, University of California, Irvine, California

Khalil H. Mancy, Professor of Environmental Chemistry, University of Michigan (Speaker)

**Butler** — The understanding of water pollution in all of its ramifications depends upon the existence of models, while the sophistication of such models depends on the availability of suitable techniques of observation. Thus a simple model might require knowledge of the presence of a single element in a system, which would be a relatively easy task, while a highly sophisticated model, beyond the capability of present technology, would require a detailed knowledge of every chemical species present in a given system.

Any concept with predictive value is a model. Thus a theory, an analogy — *e.g.* a mechanical model, and water quality criteria are examples of models commonly used. Their complexity may range from intuitive analogies to intricate computer programs.

The simplest model, involving the distribution of elemental species, is the most capable of implementation by presently available techniques. Trace element analysis is highly advanced, yet there are serious disagreements in the results of elemental analyses by several methods of determination, due to measurement problems, but also because the model may have been oversimplified. In the first case, the use of Standard Reference Materials (SRM's) offers a means to intercompare and improve measurement capabilities. In the latter case, a better understanding of the fundamental nature of a given water system will lead to better measurement techniques which will in turn provide increased quantitative knowledge.

The simple atomic distribution model can provide limited understanding, at best. The distribution of groups of atoms, the nature of distinct compounds that may be present, and the equilibria of mutual interactions must ultimately be known. The equilibria between metals and ligands are known for only a relatively few cases in clean, aqueous systems. The number of possibilities is staggering and the problem of mixed ligands has been barely treated, even in simple systems.

Kinetic models, especially those involving transport and interfacial phenomena, are at the frontier of knowledge, at present. Analytical techniques providing sound quantitative data are indispensable for understanding these problems. One of the areas of special concern is the water-sediment interface. Until such equilibria are better understood, efforts to improve many ecological problems may be meaningless. For example, phosphate release by sediments may be rate regulating rather than the rate of its introduction into certain bodies of water.

Another area of knowledge which needs further investigation is that of fundamental physico-chemical relationships in the water system. Knowledge of the thermodynamic activity of important chemical species

is very limited, yet is basic to a better understanding of many important problems. This is true both for fresh water and for the ocean, although the task in the latter case is somewhat simpler because the essentially constant salt concentration provides a medium of constant ionic strength.

Models have been explored in which biological phenomena are a part. Thus, the biological removal of phosphate no doubt involves the steps, sediment-water-algae-fish. In the last step phosphorous may return as sediment or be removed by man for use as food or for other purposes. More complicated biological processes no doubt exist and their understanding and measurement are intimately related.

Microbial catalysis is well recognized, especially in redox reactions, but the understanding is far from complete. Chemotaxis, the phenomenon of orientation of organisms by chemical species, is also of great interest. It is now recognized that bacteria have chemical senses and that the excretion of chemical substances is a means of communication between organisms, as recent work on sex attractants of insects has demonstrated.

The ways in which pollution may interfere with natural processes to provide both beneficial as well as detrimental effects have been barely explored. Pesticides are probably the best known organic substances that cause adverse biological effects, but many of the compounds that occur in oil and other organic wastes interfere with the chemical senses which keep organisms interacting in the way which is most beneficial to them. There may also be effects on the developmental biology of organisms but this has barely begun to be studied.

There are also long-term low-level effects and it is difficult to identify what is causing such effects. Pesticides are known to produce stresses at low concentrations. They decrease the insect population, thus producing a lack of food for fish. So if you use pesticides too heavily you may lose your fish, not because the fish are poisoned, but because they are starved.

We now find that heavy metals are removed and stored in animals. Thus, mercury is stored in swordfish, so we can't buy swordfish in New England any more. The removal of the phosphorus by fish has already been mentioned. These are just a few examples.

The important point I want to make is that, whether we like it or not, we make use of models in our thinking. If we are to be successful in understanding and controlling nature, we should be aware of what models we use, and of their limitations. Although a simple model like elemental composition may be satisfactory for some purposes, in other cases it may be hard to understand anything without a sophisticated biochemical-physical-ecological model.

The basic idea to remember is that everything we think about implies some model. The more aware we are of these models, and the more accu-



rately we make these models, the better equipped we will be to deal with problems that arise.

**Guinn** — Neutron activation analysis (NAA) offers a number of potential advantages for the determination of elemental pollutants in water. This technique only determines elemental composition, but it is capable of quantitatively determining some 75 elements with sensitivities equal to or better than many other conventional analytical techniques. In many cases, interferences are not a serious problem, and many samples can be analyzed using instrumental procedures in which no sample destruction or dissolution, and no chemical separations, are required.

A good practical example of the utility of the instrumental NAA method is its use as a rapid screening procedure to identify the presence of certain elemental pollutants. Even quite low levels of many elements (but often in the concentration range of concern) can be detected and measured rapidly, accurately, precisely, and nondestructively by the instrumental NAA method—in many kinds of matrices of practical importance. The determination of mercury levels in seafoods is an example of this type of application.

Instrumental NAA is found to be capable of determining mercury at the 0.5 ppm level to a precision and accuracy of  $\pm 0.02$  ppm in many kinds of biological matrices—including, for example, grains and fish. In such matrices, the instrumental form of the NAA method can usually detect mercury levels as low as 0.02 ppm. If one employs the destructive radiochemical separation form of the method, mercury levels as low as 0.0001 ppm can still be accurately measured.

Another type of application of the instrumental NAA method is in multi-element characterization analyses, in forensic studies. One example is its use to characterize the source of crude oils and fuel oils, as in the identification of the source (polluter) of an oil slick found on the surface of a body of water. We have found that a fairly simple instrumental NAA examination of a specimen of crude oil or fuel oil can detect and measure the levels of as many as 20 trace elements in the specimen. Extensive measurements have shown that the patterns of the levels of these various trace elements are characteristic of crude oils from each of the world's producing zones and fields (and also of the fuel oils produced from them). Thus, comparison of an oil slick trace-element pattern with those of oil samples taken from ships in the area can identify which one of the ships was the source of the pollution.



**Hercules** — Instrumental techniques offer many approaches to the identification of trace organic materials in water. Fluorimetry is especially attractive for the detection of traces of aromatic compounds. The recent availability of frequency-doubled laser sources should permit determinations at dilutions as low as  $10^{-12}$  molar.

The utility of electrochemical techniques should be emphasized, since they permit the determination of concentrations of ionic species and especially the thermodynamic activity, which information is often the desired parameter. Kinetic methods based on differences in reaction rate catalyzed by free and complexed metal ions, respectively, are also promising. Catalysis of chemiluminescence represents a particularly attractive possibility. Differences in oxidation state of ions can also be determined by chemiluminescence.

The role of sediments cannot be over stressed. Bottom materials, such as clays, are recognized ion exchangers and can adsorb or otherwise remove significant amounts of specific pollutants from a body of water. Long after a pollution incident may have occurred, the adsorbed material may still be present for redistribution in the water.

There are many difficult analytical problems concerned with water recycling. Present water usage is based on the concept of linear throughput from source-to-use-to-waste. Recycling, consisting of purification followed by re-introduction to the water supply, may become a common practice, and this will require highly sophisticated quality control measures. Not only must one determine the levels of substances known to be present, but constituents conceivably present must be sought. Suitable analytical methods must be developed in advance of the technology of water purification to make recycling feasible.

This symposium has been very successful in its purpose to reveal the real problems in areas of national concern, but how can the academic community respond to them? It appears that analytical chemists may be basically oriented in two ways. They may be oriented along problem-solving lines, or toward specific techniques. Both kinds of interests can co-exist within the same departmental framework. There is no fundamental conflict, and both kinds of chemists can make significant contributions.

The approach of the problem-oriented chemist is to pick out a problem and solve it by whatever techniques are necessary. The technique-oriented person would look at a list of problems to select one applicable to his particular specialty.

Perhaps the most important service an academic chemist might perform is to make his students aware of the real-life problems in a very positive way. Approaches to such problem solving may be incorporated into the

training of graduate students without any great difficulty. These could be included in special topics courses, seminars, and doctoral propositions.

It is also important that the graduate analytical chemist receive a broader background than was given in past years. This may be done by encouraging minors in biochemistry, geology, oceanography, ecology, and pharmacy, for example. Such minor studies would serve well to create an awareness and appreciation of problems needing analytical solutions.

It is unfortunate that recently created organizations such as the Environmental Protection Agency are so strongly crisis-oriented. It is granted that they are faced with many crises to which they must respond. However, this response in many respects overshadows any response in a positive way to long-term problems.

The philosophy of long-range research needs to be cultivated. The solution of the problems that may occur tomorrow but cannot be envisioned today are perhaps more important than the solution of the problems of the present.

**Sievers**—The important area of species identification in water pollution has already been pointed out. It may not be enough to know total chromium, for example, but rather whether it is present as chromium(III) or chromium(VI), because of their differing toxicities. Likewise the precise form and distribution of mercury compounds may be more important than the total mercury concentration present. Clearly, the species problem is even more complex as one moves into organic chemistry. Better methods appear to be needed for separating organic compounds, for differentiating them, or both.

Analytical chemists have hesitated to make extensive use of nuclear magnetic resonance methods for several reasons. One of these is expense, but the more significant one is that the spectra are often extremely complicated. This is true even for single compounds. When mixtures are involved, the situation has been almost hopeless.

Recent advances in nuclear magnetic resonance methodology have greatly improved its potential. Fourier transform procedures have provided increased sensitivity for NMR so that the spectra of microgram quantities or microliter samples can now be measured. Complexing techniques recently developed may be used to greatly simplify NMR spectra. For example, compounds with functional groups may be complexed with europium, which spreads out the spectra, greatly aiding their interpretation. Such procedures have been used in pesticide residue determinations and no doubt have wide applicability. The fact that hydrocarbon spectra are not shifted provides the opportunity to detect small

amounts of compounds containing functional groups in the presence of large amounts of related hydrocarbons. Probably the most effective shift reagent is  $\text{Eu}(\text{fod})_3$ , which was recently described in the literature (*J. Am. Chem. Soc.* **93**, 1522 (1971)).

Gas chromatography combined with mass spectrometry has great potential for the analysis of pollutants in water. This procedure utilizes the mass spectrometer to substitute selectivity for the highly sensitive but relatively unselective electron capture detector. Simplification of the mass spectrometer is needed to fully utilize such a procedure.

One of the problems up to the present time has been that only expensive general-purpose mass spectrometers have been produced. A simplified inexpensive instrument is needed to provide the capability of monitoring a given mass during the course of a chromatographic separation with high sensitivity. It should also be possible to change the mass detected during a separation, depending upon the retention times of the compounds of interest and the motivations of the experimenter. It would seem that such an instrument could be produced for about \$10,000 as compared with the \$50,000 to \$70,000 cost of the more versatile equipment on the market today.

The instrument suggested would sacrifice resolution for sensitivity (unit mass resolution at  $m/e$  100-200 would be adequate). The differentiation, now provided by resolution, would be obtained by using a gas chromatograph at the inlet.

An example of application is the determination of chromium or beryllium after conversion to the trifluoroacetylacetonate. The electron capture gas chromatographic procedure is relatively simple for air filter samples which are customarily ashed before analysis and quantitations at the ppb ( $10^{-9}$ ) level are achieved. No difficulties are encountered because the ashed samples are relatively free of electron-capturing interference. On the other hand, in looking for chromium at the 5-10 ppb level in biological matrices such as blood or serum, electron-capturing impurities are often encountered. These interferences can be avoided by the use of a mass selective detector.

**General Discussion** — The general discussion dealt largely with the problem of the interpretation of analytical data. Too often, the ideas as to research needs come from within pollution control agencies. The scientific community needs to inject more in-put into the development of program plans.

Analytical chemists need to present their data in terms in which it can be understood. Moreover, they cannot equivocate. Decisions must be made using the best judgement, based often on incomplete data. While this is contrary to best scientific practice, the chemist must interpret his own measurements, lest they be interpreted by others with less scientific perspective.

With respect to the reliability of specific analytical measurements, a plea was made for the more general use of Standard Reference Materials to validate procedures. There are presently only a few SRM's that are applicable and the list needs to be greatly expanded. The cooperation of water analysts and pollution technologists is required to identify the most urgent needs.





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## CHAPTER 7

# PROBLEMS IN APPLICATIONS OF ANALYTICAL CHEMISTRY TO OCEANOGRAPHY

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The general character of oceanic and estuarine systems poses special problems in applying analytical chemistry to our description of these environments. The chemistry of sea water is described in general terms and the resulting analytical problems are identified by type examples. For the more abundant constituents, analytical precision and accuracy approaching the classical atomic weight measurements is required if improved data are to be obtained. For the moderately abundant constituents and, in particular, for those elements that participate in biological processes, large time and space variations occur and the requirement of large amounts of data favors automated analyses, if precision and accuracy can be retained. For the trace constituents including many of the heavy metals, inaccuracy produced by current techniques and analysts has produced a nearly useless body of data.

Keywords: Chemical analysis; oceanography; pollution; water analysis.

## I. Introduction

Oceanography is the study of a very large, naturally occurring object, rather than a system that is arbitrarily defined for intellectual purposes. This large mass of water has been repeatedly evaporated and condensed to produce a massive leaching of the solids at the earth's surface. This process may be observed today and we see that the rates of addition and loss of compounds of the mobile elements (high aqueous solubility—little other reactivity) are millionths of 1 percent per year of the existing quantities in the oceans and even for relatively immobile (low aqueous solubility—high chemical reactivity) elements the fractional change in inventory is of the order of 0.01 percent per year. Mixing rates based on flow mea-

surements or on the distribution of cosmic ray produced radiocarbon are roughly 500 to 1000 years. The solution is well stirred and, therefore, rather uniform.

If the rates of the energetically favored reactions were rapid, with half times of decades to perhaps a century, it would be possible to write a concise thermodynamic description of the oceans and that would be that. However, many of the reactions involve complex mechanisms. Activation energies to pass through intermediates appear crucial. Some reactions appear to occur only at solid surfaces and even then at rather slow rates. Goldberg [1] estimates the rate of growth of manganese nodules, which are widely distributed in the surface sediments, at 1 to 100 atomic layers per day (0.1 mm per thousand years).

The other complicating aspect of the oceanic system is the presence of living organisms. Constituents are assimilated, the biological structure moves in contrast to the water motion and an altered distribution is produced. Swimming is important but sinking from gravitational attraction is the major process. The effects of biological activity vary from element to element depending on the degree of participation in the biological process. Productivity of the photosynthetic organisms in the oceans is limited by light and the availability of nutrients. As pointed out by Ryther [2], the situation in the ocean is rather different than on land, which he illustrates by considering the availability of fixed nitrogen. For a terrestrial environment with fertile soil one meter thick, the fixed nitrogen content of approximately 0.5 percent could support a crop or yield of approximately 50 kg of dry organic matter per meter square. The structure of the terrestrial photosynthetic organisms leads to conversion of solar energy to an organic yield of 10 g per day or an annual production of several kilograms per meter square and the local supply of nitrogen is adequate for maximum growth for many years. In contrast, the oceanic environment contains 0.00005 percent fixed nitrogen and the surface layers that have sufficient light intensity to permit plant growth (the euphotic zone) average 0.00001 percent fixed nitrogen. This productive layer is roughly 100 m thick and the reservoir of fixed nitrogen is 10 g. Complete conversion to organic matter would yield roughly 100 g of dry plant tissue. Self shading as the crop develops reduces the rate of production and the total possible crop is not achieved. The factor of 500 between the possible terrestrial yield and oceanic yield suggests that feeding the world's human population will be based on better management of the terrestrial system. Limited areas of the oceanic system have high yield because the producer extracts nutrients from a large volume of water, *i.e.*, attached plants such as kelps and turtle grass or fish that maintain themselves in areas where



upwelling continuously brings nutrient rich water into the euphotic zone. Many of the processes and phenomena studied by soil chemists and agronomists are important in the oceans but the temporal and spatial intensities of the processes are quite different.

## II. Some Historical Aspects of Oceanography

The scientific study of the oceans developed as a part of the disciplines of physics, chemistry and biology, with valid observations beginning in the seventeenth century. As noted by J. P. Riley [3] in his historical review, Robert Boyle might be considered the founder of scientific chemical oceanography in about 1670. Boyle's work included efforts to determine the salinity of sea water by evaporating samples, with results that were so variable that the method was considered unsatisfactory. Three hundred years later, this wretched characteristic of sea water salts plagues the modern analyst and we are unable to determine the simple property, total dissolved solids, by the obvious operation of quantitatively removing the water. Boyle and Hooke resorted to indirect estimates from measurements of specific gravity with a hydrometer or crude pycnometer as a more precise estimate. The use of indirect methods persists today and conductivity or refractive index measurements are employed to estimate the salinity of sea water samples.

During the eighteenth century, the first analyses of the composition of sea water were undertaken by Antoine Lavoisier, using selective precipitation during evaporation and selective extraction of the remaining salts. The fractions isolated with Lavoisier's method were shown by John Murray in the early nineteenth century to be quite impure and with the advent of the ionization theory of Arrhenius, Murray proposed that the individual "acids and bases" be determined as a sounder procedure for establishing the composition of sea water. The difficulties with Lavoisier's method that result from the multicomponent aspect of sea water are a recurring point of distress in the literature of the analytical chemistry of sea water. Analytical properties have been ascribed to one constituent and subsequently shown to be influenced by unsuspected constituents.

The development of oceanography as the integrated application of the scientific disciplines to the study of the oceans received major impetus from the planning and work undertaken on the world-wide expedition of H.M.S. CHALLENGER from 1873 through 1876. The analyses of samples from this expedition by W. Dittmar [4] were reported in a de-

lightly comprehensive report. The care and logic with which Dittmar devised and tested analytical procedures and his realistic appraisal of the probable accuracy and precision of the sample results are a model of quality that modern analysts have difficulty in emulating. Considering the limitations of chemical theory and analytical techniques available to Dittmar, his results are a remarkable achievement. The accuracy of his results as judged by comparison with modern results is better than 1 percent except for the magnesium results that appear to be 2.5 percent too high.

The methods for each constituent were tested and then adhered to rigidly, so the systematic errors might be nearly constant and Dittmar hoped that if subsequent investigations demonstrated systematic errors, a simple correction might be applied to his results for the samples from all the oceans. In addition, the precision of the measurements was estimated to be of the order of 0.1 percent. Dittmar's data show variations that are larger than would be expected to arise by chance, but he could not correlate them with depth or geographical position except for calcium and the calcium/chloride ratios for deep waters were found to be larger than the ratios for surface waters. The calcium variation has been "rediscovered" every few decades since Dittmar's publication.

The results for the 77 samples studied by Dittmar established that the major constituents of sea water are present throughout the oceans in nearly constant proportions, but that small variations might be present. This principle of constant proportions has been used as a major feature of oceanographic work. Each of the major constituents may be estimated from any one of the others when the proportionality constants have been determined. Many of the papers on chemical oceanography during the twentieth century have been studies of the proportionalities, usually in the form of the ratio of a constituent to chlorinity (the sum of the halides expressed as chlorine equivalent using 1900 atomic weights—see below). The importance of these results is that all the physical properties, and, in particular, density, may be expected to correlate to chlorinity with high precision.

Salinity was estimated by Dittmar as the sum of the individually determined constituents, a laborious procedure. The need for precise relationships between an easily determined quantity and both salinity and density (more accurately, specific gravity) led to the formation of an international commission in 1899 to investigate and advise on the definition and determination of salinity. The work of the commission was reported by Forch, Knudsen and Sørensen in 1902 [5]. Sørensen devised a method for evaporating sea water samples that yielded a reproducible mass of residue, a tedious procedure but one that the commission deemed ac-

ceptable. The analytical results from Sørensen's method were taken as being identical with a definition of "salinity as the weight in grams of the dissolved inorganic matter in 1 kg of sea water, after all the bromide and iodide have been replaced by the equivalent amount of chloride, and all carbonate converted to oxide." The results with Sørensen's procedure are 0.46 percent smaller than the mass of total dissolved salts in open ocean waters and the precision can be 0.05 percent, *i.e.*, the accuracy is an order of magnitude poorer than the precision. The method was used on nine samples, of which two came from the open ocean and the others came from the North Sea, Baltic Sea and Red Sea. A correlation between measurements of chlorinity and salinity by the Sørensen procedure was found and the best fit equation was

$$S = 0.03 + 1.805 [Cl] \text{ in g/kg or } \text{‰} \quad (1)$$

The commission felt that "salinity" could be estimated reliably from either specific gravity or chlorinity measurements, but their report clearly distinguishes between directly measured values of salinity and estimates from another quantity. As Knudsen points out in the first sentence of the section of the report dealing with salinity, "By salinity one means when no other stipulations are made, the weight of dissolved salt per unit weight of sea water." Knudsen used the symbol  $S_{Cl}$  to designate values derived from chlorinity measurements, but others have felt that this was too cumbersome and nearly all the twentieth century oceanographic literature conforms to the use of the symbol  $S$  and the word salinity to mean the Sørensen-salinity estimated from chlorinity. It is necessary to guess the meaning of salinity in various publications and it appears probable that authors using ordinary dictionaries and without special knowledge of oceanographic nuances would mean total dissolved salts and that authors publishing in oceanographic journals would mean the above peculiar quantity.

During the past two decades, instruments for measuring relative conductivity of sea water samples with a precision of 0.005 percent have been devised and are commercially available. The high precision results from the use of inductively coupled cells rather than metallic electrodes with their instability. The superficial simplicity of the manipulations required in the use of such instruments has led to great popularity. There is a serious deficiency in the conductivity technique, which is that reproducible and defined standards are not available. An adequate standard should be defined to 0.001 percent and should be reproducible to the same extent. Current standards for electrolytic conductance based on solutions of

potassium chloride are reproducible only to approximately 0.04 percent. In lieu of an adequate standard, water samples from the ocean are being used as a reference substance. An effort at building an instrument to determine the absolute conductivity of aqueous solutions has been initiated at the National Physical Laboratory, but, to my knowledge, has not been successful.

This deficiency associated with conductivity measurements has led to some unfortunate developments and misconceptions in the recent literature. The problem has been considered by the UNESCO Joint Panel on Oceanographic Tables and Standards and tables [6] of recommended values of relative conductivity have been distributed. In addition, a note that encourages the use of these tables has been published in several journals, [7] as an example. However, the note is titled "redefinition of salinity" and includes the statement "Expression (3) constitutes the recommended definition of salinity" and the referenced expression is

$$\begin{aligned} S^{\circ}/_{\infty} = & -0.08996 + 28.29720 R_{15} \\ & + 12.80832 R_{15}^2 - 10.67869 R_{15}^3 \\ & + 5.98624 R_{15}^4 - 1.32311 R_{15}^5 \end{aligned}$$

$R_{15}$  is the ratio of the conductivity of a water sample to that of water having a salinity of exactly 35‰ both liquids being at a temperature of 15 °C and under a pressure of one standard atmosphere.

Examination of this proposed "definition" shows some remarkable features, not the least of which is the fact that it is illogical. Water with little conductivity would have a negative salinity, a difficult concept to comprehend. The definition seems mysterious unless there is some place some water with a "salinity of exactly 35‰" that is known to the authors but not generally known. The reasons that the measurements of Cox *et al.* [8] produce this strange empirical relationship seem to be the following.

1. Salinity was not measured, but rather estimated from chlorinity measurements. The salinity estimates were made using the expression  $S = 1.80655 [Cl]$  rather than the relationship of Knudsen  $S = 0.03 + 1.805 [Cl]$ . The Knudsen results had been criticized by Cox [8] as implying that salinity was nonconservative in the ocean, *i.e.*, if waters of different salinity and chlorinity were mixed, the salinity and chlorinity of the mixture could not be computed by simple proportionality.



2. Neither expression for estimating salinity from chlorinity has universal validity. In the open ocean, chlorinity and presumably salinity vary because of evaporation and precipitation of nearly pure water and there should be direct proportionality between chlorinity and salinity. The functional form of Knudsen's empirical relationship is due to the distribution of the samples, which came in a large part from areas where substantial mixing of sea water with land runoff had occurred. Since river waters draining into the Baltic Sea contain relatively large concentrations of salts as compared to chloride, the empirical expression predicts appreciable salinity when the chlorinity is zero. Each expression seems to have a legitimate application but the correct one must be identified in each situation.

3. The samples on which the new tables and definitions are based included low salinity waters from the Baltic Sea. The equation used to estimate salinities for these samples will produce an underestimate of approximately  $0.1\text{‰}$  and the negative intercept arises entirely from the manipulation of the data.

Most chemists find the mathematical transformation of the chlorinity to estimates of salinity not very useful. For example, Carter *et al.* [9] in 1933 stated "The salinity is ascertained from the determined chlorinity by reference to the Hydrographic Tables, or in other words, the chlorinity is multiplied by a constant and 0.03 added to the resulting value. The term salinity thus seems to be superfluous and its determination is a useless gesture. The direct reporting of the nature of sea water as the chlorinity appears to be the easier and more logical method." For several decades, workers on the West Coast of the United States and Japan reported only chlorinity. Now everything is converted to Sørensen-Salinity and the several hundred thousand values recorded in the National Oceanographic Data Center are a mixture of **estimates** from chlorinity titrations or conductivity comparisons.

There is a deep-seated desire by many physicists and biologists who study the ocean to know the "saltiness" of various parts of the system. The fact that there is no adequate method of measurement does not abate that desire, and it does not appear to be possible to abandon the uncertain estimating process described above. This is an area where analytical chemistry is the paramount tool and a new technique with the required precision and simplicity would find wide acceptance.

### III. The Determination of Halides

The most numerous analyses of sea water have been the titrimetric estimation of the halides, using precipitation with silver nitrate solutions. The work of the international commission [5] reported in 1902 put the measurement of halides on a sound basis. The commission recognized the extreme importance of an adequate standard and initiated the distribution of ampoules of "standard sea water," which are samples of sea water that have been compared with the original standard water using the Volhard method by weight to obtain a precision of 0.003 percent. This practice has continued to today and we are in the very pleasant position of having several hundred thousand observations connected to a precise standard in an unequivocal manner.

The chlorinity was defined in 1902 as the mass of chlorine equivalent to the total mass of halogen in the mass of 1 kilogram of sea water. By 1937 the original stock of standard water was nearly depleted, and Jacobsen and Knudsen [10] undertook the preparation of a new supply. They recognized that the definition of chlorinity was dependent on the atomic weight values and, therefore, subject to change with changes in the accepted values. Since the purpose of chlorinity observations on sea water is to describe the oceans and to compute the specific gravity from empirical relationships, Jacobsen and Knudsen recommended that the chemical concept of chlorinity be discarded and be replaced by an oceanographic operational definition  $Cl = 0.3285234 [Ag]_{\text{‰}}$  or "the number giving the chlorinity in per mille of a sea-water sample is by definition identical with the number giving the mass with unit gram of Atomgewichtssilber just necessary to precipitate the halogens in 0.3285234 kilogram of the sea-water sample." This gives rise to the unusual situation where the symbol Cl refers to a mass of silver.

It seemed impractical to change the data collected over nearly four decades and the new definition was made to be numerically identical to the equivalence between silver and chlorine that had been assumed by Sørensen in 1902. The specification to seven significant figures means that this arbitrary definition will not need refinement. The ratio of chlorine-equivalent to chlorinity of approximately 1.0045 is easily handled when problems requiring chemical concepts are considered. This procedure of putting chlorinity measurements on a "silver standard" seems eminently sound.

The only point of real difficulty in the past seven decades has been the limited use of sodium chloride as a standard by a few observers, as pointed out by Reeburgh [11], and these results can be converted to the

chlorinity definition if the assumed atomic weights have been stated by the authors. However, there is an aspect in which some confusion has arisen. In the review by Carritt and Carpenter [12], we pointed out that many of the observations of ionic ratios showed discrepancies from constancy that were larger than might be expected from the estimated precision of the procedures and a statistical evaluation suggested that small variations might be present in the oceans—similar to Dittmar's conclusions. These comments, in part, stimulated an extensive investigation of specific gravity, relative conductivity, chlorinity and individual element concentrations for 205 samples collected throughout the oceans. The first measurements on these samples were reported by Cox *et al.* [13] and it was concluded that the density (actually specific gravity) of sea water samples could be estimated from conductivity measurements with less variation than from chlorinity measurements and this conclusion received considerable publicity. Subsequently, as reported by Cox *et al.* [8], the precision of the procedure for chlorinity measurement was found to be less than had been thought. An alternate procedure was used to repeat the chlorinity determinations and "comparison of the results shows that a large part of the scatter previously reported in the relationships between chlorinity and conductivity, and between chlorinity and density, was due to analytical errors."

This erroneous apparent failure of the constancy of major constituent proportions has led to suggested rejection of chlorinity as a primary measurement. For example, Horne [14] states "the electrical conductivity is a property which can be measured with very great accuracy both in the laboratory and *in situ* . . . The concept of chlorinity has outlived its usefulness and should be allowed to die a natural death before it becomes an impediment." It appears to me that Horne was misled into confusing accuracy with precision and the "silver-standard" chlorinity will remain the property of sea water which can be measured with a precision of 0.003 percent with the Standard Sea Water Service Volhard technique when needed and with a definition that makes accuracy meaningful only in terms of the purity and isotopic composition of the standard silver. Until a technique for determining the cell constant of conductivity instruments is devised, careful chlorinity measurements will serve a useful and essential function in the study of the oceans and in the calibration of conductivity instruments.

The requirements of extreme precision in oceanographic measurements derive from the monotony of the distribution of properties in the oceans. Rather easily observed variations of the motions and saltiness occur in the surface layers to depths of 200-400 m (5 to 10 percent of the volume). For

the remainder of the system, variations with time and position are rather small. For example, some data being studied by W. Boicourt at the Johns Hopkins University and the Woods Hole Oceanographic Institution are plotted in Figure 1. The apparent change in S and  $O_2$  with time might be construed as an oceanographic event. The change in S is approximately 0.1 percent of the mean value and the change in  $O_2$  is approximately 4 percent of the mean value. Assurance that the precision of such measurements over the extended period of time was 0.01 percent for S and 0.4 percent for  $O_2$  is needed for confidence in interpretation.

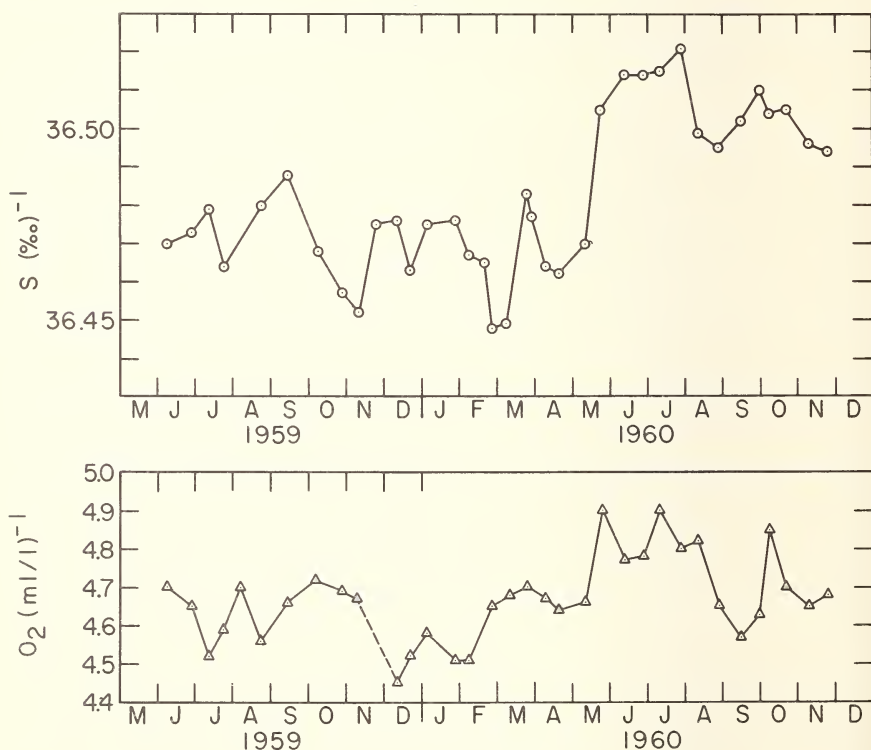


Figure 1. Time record of salinity and oxygen on the 18°C isotherm at Panulirus Station (32°10'N 64°30'W).

#### IV. Chemical Composition of Sea Water

Some approximate average values for the total concentrations of the various elements in sea water are listed in Table 1. The elements have



Table 1. Approximate sea water chemical abundances.<sup>a</sup>

Element	mg/l	Element	mg/l
Cl	19,000.	Sn	0.003
Na	10,600.	Pb	0.003
Mg	1,300.	U	0.003
S (SO <sub>4</sub> )	900. (2600)	V	0.002
Ca	400.	Mn	0.002
K	380.	Ti	0.001
Br	65.	Th	0.0007
C (HCO <sub>3</sub> )	28. (140)	Co	0.0005
N <sub>2</sub>	13.	Ni	0.0005
O <sub>2</sub>	8.	Ga	0.0005
Sr	8.	Cs	0.0005
B	4.8	Sb	<0.0005
Si	3.	Ce	0.0004
F	1.3	Hg	0.0003
A	0.6	Y	0.0003
N (NO <sub>3</sub> )	0.5 (2)	Ne	0.0003
Li	0.2	Kr	0.0003
Rb	0.12	Ag	0.0003
P (PO <sub>4</sub> )	0.07 (0.2)	Bi	0.0002
I	0.05	Cd	0.00011
Ba	0.03	W	0.0001
Al	0.01	Ge	0.0001
Fe	0.01	Xe	0.0001
Zn	0.01	Cr	0.00005
Mo	0.01	Sc	0.00004
Se	0.004	Be	0.00005
Cu	0.003	Nb	0.00001
As	0.003	Tl	<0.00001

<sup>a</sup> Modified from Goldberg [1].

Table 1. Approximate sea water chemical abundances.<sup>a</sup> (continued)

Element	mg/l	Element	mg/l
He	0.000005	Dy	$2 \times 10^{-7}$
Au	0.000004	Ho	$8 \times 10^{-8}$
La	$3 \times 10^{-6}$	Er	$2 \times 10^{-7}$
Ce	$4 \times 10^{-7}$	Tm	$4 \times 10^{-8}$
Pr	$2 \times 10^{-7}$	Yb	$2 \times 10^{-7}$
Nd	$8 \times 10^{-7}$	Lu	$4 \times 10^{-8}$
Sm	$1 \times 10^{-7}$	Ra	$3.0 \times 10^{-11}$
Eu	$4 \times 10^{-8}$	Pa	$2 \times 10^{-12}$
Gd	$2 \times 10^{-7}$	Rn	$9.0 \times 10^{-15}$

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<sup>a</sup> Modified from Goldberg [1].

been grouped into four ranges of abundances, which might be termed "major, minor, major trace and minor trace." Some very rough generalizations may be made concerning spatial variations in the abundances of the elements. As indicated above, the major elements occur in nearly constant relative proportions and the variations may be of the order of a few tenths of one percent. The minor elements show greater variation, with the extent of variation correlating with the participation of each constituent in biological processes. Bicarbonate ion, molecular oxygen, silicate ion and nitrogen as primarily nitrate ions show the greatest variation. For the other minor constituents, the variations may be approximately 1 to 10 percent. The major trace elements are mostly metals and many are associated in biological processes. Phosphorus as phosphate shows extreme variation but the others may show variations of a factor of two to six. The data for the minor trace elements are so sparse and uncertain that no impression of the real variability can be derived from the existing information. These rough estimates of the variations may serve as a guide in developing analytical methods.

## V. Distinctive Aspects of Sea Water Analysis

In contrast to most chemical analyses that are made for some immediate purpose, analyses of sea water provide data that may be referenced repeatedly. The data that have been accumulated over an extended period of time and observations by more than one group are combined to produce descriptions of various ocean regions. While there are many examples of research carried to completion by a single individual during a period of a few years, much oceanographic research is based on pooled data. Questions are posed after the data have been collected, and the analyst is not provided with a clear estimate of the required accuracy and precision of the measurements. Because of this aspect, the compromise that must be made between accuracy, precision, cost, speed and ease in choosing an analytical method should be weighted toward accuracy and precision. For example, it is justifiable to build a mass spectrograph for dissolved gas analyses if the accuracy or precision of the measurements would be improved over those obtainable with commercial instruments.

Sample integrity is particularly significant. Water samples from the ocean are several hours old when they arrive on the deck of the research vessel. Contamination of the sample with materials from the walls of the sampling equipment is possible. Samplers must be lowered open and inadequate flushing at depth may lead to contamination with surface waters, a potentially serious problem for materials with a strong vertical gradient such as radionuclides supplied to the ocean by atmospheric fallout. Hydrophobic coatings have only recently been used to decrease reaction of the sampler surfaces with the sea water.

Once on deck, reduced pressure and increased temperature may lead to loss of gases and special samplers are necessary for samples that are analyzed for gases. A striking temperature and pressure effect has been recently demonstrated for ion exchange equilibria of such ions as potassium in the interstitial waters of sediments and a large part of the apparent enrichment with potassium in such waters has been shown to be a sampling artifact.

Transfer of sample aliquots to smaller containers provides increased surface areas relative to the aliquot volumes. Adsorption of dissolved and particulate material on the walls of the sample container provides an enriched environment for the rapid growth of bacteria. Biological processes can change the sample composition within a few hours. Rapid freezing has been shown to stabilize the phosphate and nitrate content for several months but changes in ammonium content have been observed with

storage at  $-20^{\circ}\text{C}$ . Shipboard analysis is obviously desirable and, at a minimum, the sample must be processed to some stable form. Plastic containers have been used with serious evaporative losses through low density polyethylene bottles and nearly complete uptake of phosphate on the walls of polyethylene bottles. Most of the organic constituents and the "heavy" metals occur at micromolar or less concentrations and sampling contamination or losses must be evaluated in detail for each procedure.

Transport of samples to shore laboratories aggravates the sample integrity problem, but shipboard analysis poses problems in equipment and analytical soundness. Shipboard laboratories are subject to various amplitudes and frequencies of motions, ranging from vibrations from reciprocating engines to the pitching and rolling of the whole ship. Simple manipulations involved in analytical procedures take on new dimensions when they are performed with one hand while seasick and using the other hand for physical stability. Measurement techniques have been limited to volumetric titrations, spectrophotometry, fluorometry and electrometric determinations using solid electrodes. As better ships have been made available, more elaborate equipment such as mass spectrometers for nitrogen-15 tracer experiments are being used successfully. Most of the routine methods that are used on shipboard are spectrophotometric because of the reliability of the equipment and the simplicity of manipulations. These procedures are adaptable to automatic equipment and a modified six channel AutoAnalyser was used on a recent expedition to the Black Sea.

## VI. Some Examples of Analytical Problems

The major problems that may be found in using the chemical oceanographic data are inaccuracies and the lack of adequate tests of procedures for the purpose of making realistic accuracy statements. As noted above, many oceanographic questions could be approached using relative values if adequate standards could be devised. Mangelsdorf and Wilson [15] have developed a technique of difference chromatography using ion exchange membrane cells as a sensor that is responsive to small variations in the composition of solutions passing through the column. Using Standard Sea Water as an arbitrary reference, they have been able to examine several hundred samples for variation in the proportions of sodium, magnesium, calcium, potassium, sulfate and chloride. The precision of their measurements range from 0.1 percent for magnesium to, on occasion,



0.02 percent for potassium, *i.e.*, the technique is suitable for oceanic research. The results for potassium, which had not been studied previously with precise techniques, showed that the variations in the relative concentrations of potassium are approximately 0.1 percent. Measurements by their technique which require stability of fractions of a microvolt in the detecting circuit cannot be considered routine, but the high data rate associated with the multicomponent response of the system makes it attractive for oceanic surveys based on large numbers of samples. The limitations are that all the samples must be examined with reference to the same standard and only relative values are obtained.

#### A. MAGNESIUM

Other studies of the major constituents have been based on "absolute" measurements, using supposedly known solutions as standards or reference solutions for tests of gravimetric procedures. The tediousness and low data rate of the methods for the major constituents have prevented real delineation of variations in the oceans. The results for magnesium may be used to illustrate the problem.

Some of the magnesium analyses of sea water are summarized in Table 2. The work of Culkin and Cox was based on 66 samples collected throughout the oceans and is the most comprehensive of the recent work. However, there appears to be a systematic error in the titration procedure used by Culkin and Cox, which is that the photometric end point that they used is approximately 1 percent beyond the equivalence point. This point is discussed by Manella [16] and the results of calculations to evaluate the possible error are shown in Figure 2. Correction of the results of Culkin and Cox for this probable error would change the Mg/Cl ratio from 0.06692 to 0.06629, in close agreement with Manella's result of 0.06626. The other titrimetric results probably have end point errors of 1 percent or more. The results of Billings *et al.* were corrected approximately from 0.0675 mg/l per ‰ to 0.0658, assuming a density of 1.025 g/cm<sup>3</sup>.

As may be seen in Table 2, it is difficult to come to any conclusions regarding possible differences in the magnesium content of various parts of the oceans. The data reported for the Pacific Ocean average roughly 1 percent higher than data for samples from the Atlantic Ocean, but neither Dittmar nor Culkin and Cox, who studied samples from both oceans, found systematic differences between these oceans. It appears that there are unsuspected errors in the analytical methods as used by the various authors.

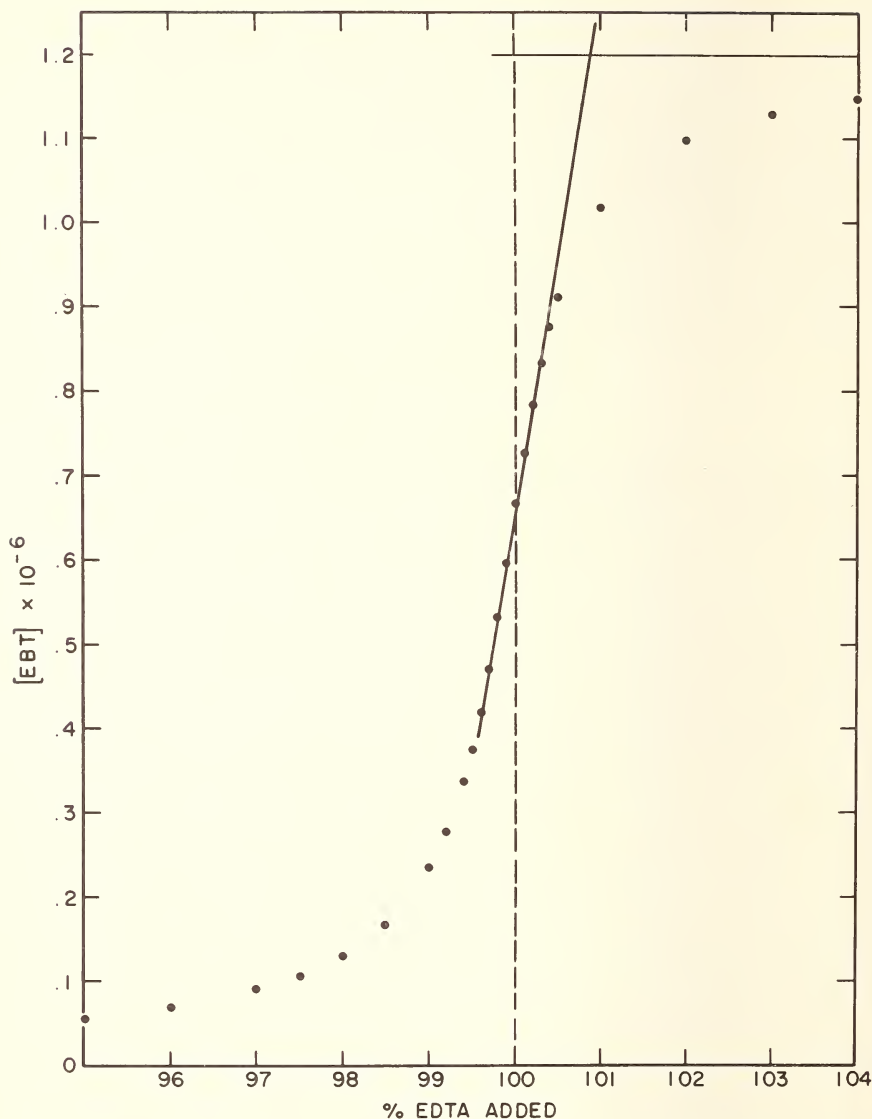


Figure 2. Theoretical titration curve for the analytical system Mg-EDTA-EBT used by Culkin and Cox (1966).

Billings *et al.* report a 10 percent temporal variation in the magnesium to chlorinity ratio for a station near Bermuda. Samples from the same area studied by Manella did not show variations in the ratio larger than 0.1 percent, and it may be inferred that there are substantial errors in the atomic

Table 2. Observations of magnesium—chlorinity ratio.

Reference	Locality	Method	Mg/Cl
Dittmar (1884)	Various	1	0.06801
Thompson and Wright (1930)	N. Pacific	1	0.06695
Voipio (1957)	Baltic	2	0.06693
Voipio (1959)	Barents	2	0.06742
Pate and Robinson (1961)	Pacific	4	0.06689
Culkin and Cox (1966)	Various	3	0.06692
Szabo (1967)	Atlantic	6	0.06650
Tsunogai, et. al. (1968)	N. Pacific	5	0.06676
Billings, et. al. (1969)	Atlantic	7	0.0658
Manella (1971)	Atlantic	8	0.06626

1. Gravimetric as magnesium ammonium phosphate.
2. Titrimetric difference. Total alkaline earths determined with EDTA standardized against calcium salt with visual end point.
3. Titrimetric difference. Total alkaline earths determined with EDTA standardized against calcium salt with photometric end point.
4. Titrimetric difference. Total alkaline earths determined with EDTA as the primary standard and visual end point.
5. Titrimetric difference. Total alkaline earths determined with EDTA standardized against zinc metal with visual end point.
6. Titrimetric difference. Total alkaline earths determined with EDTA standardized against magnesium metal with visual end point.
7. Atomic absorption.
8. Magnesium isolated with ion exchange and titrated with CDTA standardized against magnesium metal with photometric end point.

absorption procedure, even though the precision with standards was 1.5 percent.

Finally, several of the reports listed in Table 2 claimed precision and, presumably accuracy, of a few tenths of one percent. It appears probable that there were determinate errors of approximately 1 percent in these results. The error is just large enough ( $10^{19}$  g Mg in the oceans) to affect work on the colligative properties of sea water and the ionic balance.

## B. STRONTIUM

There are not a large number of studies of the strontium content of the oceans, but strontium may be used to show the character of the data for a minor constituent. The first quantitative estimates were by Webb [17], who reported 13 mg/kg with an emission spectrographic method in the late 1930's. Flame photometry was popular in the late 1940's and measurements by Odum [18] and others led to values of 8.2 to 8.5 mg/kg. Application of atomic absorption in the 1960's by Angino *et al.* [19] led to values of 7.2 to 7.5 mg/kg. One explanation would be that the strontium content of the oceans is decreasing approximately exponentially with time. However, Carr [20] has shown that the procedure of calibrating the atomic absorption measurements with standards prepared in sodium chloride solutions with a total salt content different from that of the samples produces systematic error. Comparison of internal standards or matched synthetic standards with the previous standards showed the effect to be approximately 15 percent and the results of Angino *et al.* differ from the older ones by an amount that may be accounted for almost entirely by the improper standardization.

Turekian and Schutz [21] used x-ray fluorescence on freeze-dried sea water samples from all the oceans and found differences of 4 percent between the Atlantic and Indian oceans, but their precision was 2 percent. They report an average value of 7.5 mg/l of strontium at a chlorinity of 19.00‰ but feel their values are low because the "values probably have a systematic error in the standards." Andersen and Hume [22] have analyzed samples from the Atlantic using flame photometry following concentration with an ion-exchange procedure. They report an average value of 7.5 mg/l and feel their results are accurate with a precision of 0.5 percent. It appears that the Sr/Cl ratio in the oceans may vary by a few percent and clear description awaits application of methods with accuracy and precision of 0.1 percent.

## C. DISSOLVED OXYGEN

The number of measurements of dissolved oxygen on samples from the oceans is second only to the chlorinity or salinity estimates. Most of the determinations have been made using the Winkler [23], titrimetric method, for which the manipulations are disarmingly simple. Usually the novice is given the job of carrying out the procedure and a precision of



roughly 1 percent is fairly easily achieved. There is a large variation of dissolved oxygen in the ocean, with values ranging from 8 ml/l at the surface at high latitudes to near zero in many cubic kilometers at intermediate depths in the Pacific Ocean. The dissolved oxygen concentrations are related to the intensity of biological processes and the rates and patterns of physical motion, and are of general interest.

It might be expected that methods with a precision and accuracy of a few percent would be entirely adequate for delineating the distribution of dissolved oxygen in the oceans. However, many interesting aspects of the oceans might be revealed by small variations in the dissolved oxygen concentrations. For example, exchange between the atmosphere and ocean occurs with small gradients and disequilibrium of more than approximately 5 percent is rarely observed. Errors of 2.5 percent would change the flux estimate by a factor of 2 and the errors in the dissolved oxygen concentration data make oxygen balance computations very uncertain.

Measurements of dissolved oxygen in the North Atlantic Ocean during the middle 1930's have been compared with measurements made during the 1950's with the result that there was an apparent change in the deeper waters of the North Atlantic during the two decades. The apparent rate of change would be compatible with the hypothesis that the deep waters were saturated with dissolved oxygen around 1810, a period of extreme cold. This leads to the radical idea that the North Atlantic had been mixed and saturated abruptly at that time and that there had been a slow decrease since that time. Subsequent study of the procedures used suggests that rather than a catastrophic overturn in the North Atlantic, the spectacular turnover was in personnel on the expeditions. The Winkler method was modified to be more similar to that described in standard texts for iodometric estimation of copper in metallic samples. The associated errors from air oxidation of iodide are negligible for decinormal solutions of iodine that are used in copper assay, but many percent for millinormal solutions that are involved in oxygen analyses of sea water. Similar discrepancies between British and American expeditions may be accounted for in terms of the excessive acidity required in the use of dichromate as a standard substance.

The reason that the dissolved oxygen data are uncertain is that solutions of oxygen in water have not been used as standards. The Winkler method involves reacting the dissolved oxygen with manganous ion and subsequent reaction of the oxidized manganese with iodide to produce iodine, which is frequently estimated by titration with thiosulfate. The unstable thiosulfate solutions have been standardized with iodine that is generated by reacting iodate or chromate with iodide. The method may be

used with an accuracy of 0.1 percent if the various errors are minimized, as reported by Carpenter [24]. However, errors of many percent may be easily introduced. A frequent error is associated with the volatility of iodine, and it would be unfortunate if the oceans appeared to lose several percent of its oxygen content simply because of the availability of magnetic stirring devices which aggravate the loss of iodine during titrations. With reference to the data plotted in Figure 1, the apparent time trend may be real and the correlation between the salinity and oxygen data tends to reinforce that idea, but the nagging possibility of systematic errors introduced by changes in technique is difficult to eliminate.

A variety of methods for oxygen analysis has been introduced in the past two decades. Gasometric methods have been restricted due to difficulties in manipulations in shipboard laboratories. The gas chromatographic technique developed by Swinnerton, *et al.* [25] has proved to be practical for shipboard use. The dissolved gases in a sea water sample are stripped into the carrier gas flow in a simple glass chamber and carried directly into a conventional gas chromatograph for analysis. The field performance of the technique was studied by Swinnerton and Sullivan [26], who concluded that the technique was more rapid and simple than previous methods and the precision was comparable to other methods. They found systematic differences of approximately 5 percent between the gas chromatographic results and analyses by the standard Winkler method and note that "the discrepancies are at present unexplained." Further comparisons using a revised Winkler procedure showed agreement between gas chromatographic and titrimetric results of better than 1 percent. Some of the reasons for the previous discrepancy are discussed by Carritt and Carpenter [27].

An attractive feature of the gas chromatographic technique is that results for total carbon dioxide, nitrogen and argon can be obtained with modification of the procedures. Mass spectrometry also has the virtue of versatility, with the added possibilities of extreme sensitivity. As shown by several groups at the Scripps Institution of Oceanography, mass spectrometric techniques can be used for the noble gases at extremely low concentrations. In addition, information on the isotopic composition may be essential in distinguishing between biological and physical processes in the ocean, as illustrated by Rakestraw, *et al.* [28]. The oceanographic applications of analyses for the several gases have been outlined by Benson [29]. Mass spectrometric techniques appear to be most powerful tools for dissolved gas analyses, but suffer from economic and data rate considerations. It appears probable that such techniques will receive increased attention for use in specialized research projects.

Polarographic techniques for dissolved oxygen determinations have received sporadic attention. Enthusiasts have tolerated the problems of the dropping or streaming mercury electrodes under field conditions or struggled with the poisoning of solid electrodes. The membrane solid electrode in either the polarographic or galvanic modes has received some application (Carritt and Kanwisher/[30], and Mancy *et al.* [31]). Such electrodes solve many of the poisoning problems, but have remarkably large temperature coefficients, sensitivity to ambient fluid motion and light, and changing response with time. My own personal experiences suggest that such devices might have application as thermometers, current meters, photometers or clocks and, in fact, an electrode has been used to sense weak motions in the deeper portions of the ocean. The principal application of membrane electrodes to oceanographic dissolved oxygen determinations has been in the form of recording continuous vertical profiles for the study of details in the distribution, which is an application where even relative values are useful. The quality of the data collected with dissolved oxygen electrodes depends critically on the skill of the observer and attention to frequent standardization, so that no generalization about the accuracy of such techniques can be made.

The introduction of these various methods for dissolved oxygen analysis has added versatility to the oceanographic field, but will make it more and more difficult to assess the probable accuracy of the data.

#### D. ZINC

The current state of the knowledge of the major trace constituents may be illustrated by considering data for the zinc concentrations in the ocean. Errors may be expected from both contamination and loss when constituents at the micromolar concentration level are sought. Robertson [32] presents data on the role of contamination in trace element analysis in sea water. As he shows, zinc is ubiquitous and may appear in the terminal analysis from the structural materials used in sampling, sample containers, solvents, and reagents. Adoption of a particular technique such as neutron activation attenuates some of the problems but does not eliminate them as has been frequently overstated. No generalization concerning losses from the sample can be made, except that many investigators have neglected to study the significance of this factor for their particular experimental conditions. The availability of radioactive isotopes of so many of the elements makes such oversights almost inexcusable. Many of the con-



tamination and loss effects are functions of sample size, surface to volume ratios, *etc.* and would be detected if these variables were studied. Replication to produce computed internal blanks, both positive and negative, would add considerably to the quality of the data.

Dr. Derek Spencer at the Woods Hole Oceanographic Institution has attempted to assess the intensity of physical and biological processes that cause spatial variation in the distribution of many of the major trace elements in the ocean. Variations of perhaps a factor of two or so are expectable. Concentrations that have been reported in the literature vary by more than an order of magnitude. Is the knowledge of the oceans as represented by Spencer's model missing an important concept or intensity of some process or are the analytical data misrepresentations?

Some insights into this problem can be found in the recent study by Brewer and Spencer [33]. As they state in their introduction, . . . "the concentration levels of most trace elements in sea water has been, and still is, a matter of some controversy. This study was organized as an attempt to determine whether different laboratories, working independently, could obtain the same values for these various trace elements in two samples of sea water." The results from the various laboratories for zinc concentrations are shown in Table 3. Brewer and Spencer discarded four of these values and computed a mean for sample 1 of  $4.92 \mu\text{g/l}$  and a standard deviation of  $4.60 \mu\text{g/l}$  and, for sample 3, the mean was  $5.6 \mu\text{g/l}$  and the standard deviation was  $5.3 \mu\text{g/l}$ . Sample 1 was Pacific Deep water and sample 3 was Caribbean Deep water. The most elemental question might be concerned with the existence of a difference in the zinc concentrations at the two positions, but such questions are clearly beyond the capabilities of present practices. No information that would lead to a preference for a particular method can be gleaned from these data. Equally disastrous results were achieved with a few thousand dollars worth of equipment and with devices costing millions of dollars.

One of the possible reasons for differences in the results with different methods could be that some techniques measure only the ionic form and others the total zinc. The scatter is so large that such effects cannot be detected in the zinc data. The authors note that "the total methods gave significantly **lower** results for cobalt than the extraction methods."

One point seems worth noting. Brewer and Spencer imply that laboratories are being compared. In a similar vein, Carritt and Carpenter [27] describe a comparison of modifications of the Winkler method for dissolved oxygen. In fact, the analytical results obtained by different people are being compared—some with perhaps years of experience and some with almost none. While development of new methods will contribute to



Table 3. Results of intercalibration of analyses for zinc reported by Brewer and Spencer [33].

Lab	SAMPLE 1 Result $\mu\text{g/kg}$	SAMPLE 3 Result $\mu\text{g/kg}$	Method
1501/28/07	0.02 0.6 1.3	5.2 6.1 6.6	Dithizone extract into chloroform. Colorimetric
1619/28/05	4.5 4.5 4.7	6.7 5.4 6.3	Solvent extraction APDC-MIBK. Atomic absorption
0800/04/01	4.2 5.1 3.5	6.2 6.4 6.2	Neutron activation of dried sea salts
1105/10/07	5.2 4.0 5.0	5.8 6.6 7.0	Solvent extraction APDC- chloroform. Atomic absorp- tion in methanol solution
1200/07/07	6.11	3.90 3.58	Adsorption chelex-100- Eluted $\text{HNO}_3$ Solvent extraction APDC-MIBK X-ray fluorescence
1600/10/07	4.8	6.6	Solvent extraction APDC- MIBK. Atomic absorption
1500/16/07	99.15 1.00 1.25	1.47 1.86 1.72	Solvent extraction APDC- MIBK. Atomic absorption
1200/06/06	0.65 1.85 2.15	2.95 2.95 2.90	Solvent extraction APDC- MIBK. Atomic absorption
1650/20/07	3.9 3.3 2.9	5.2 3.9 4.1	Solvent extraction APDC- MIBK. Atomic absorption
1740/08/08	4.2 3.9 3.0	5.5 5.3 5.6	Solvent extraction. Dithizone Colorimetric

Table 3. Results of intercalibration of analyses for zinc reported by Brewer and Spencer [33] (continued).

Lab	SAMPLE 1	SAMPLE 3	Method
	Result $\mu\text{g/kg}$	Result $\mu\text{g/kg}$	
1253/23/08	4.8	44	Neutron activation of freeze dried sea salts
	6.2	5.6	
	4.7	5.2	
0000/01/09	6.0	6.8	Neutron activation of freeze dried sea salts
	5.3	8.0	
	6.8	6.5	
8/07/70	0.8	0.75	Neutron activation of freeze dried sea salts
	0.76	0.72	
18/9/70	18.5	22.8	Adsorption chelex-100 Eluted $\text{HNO}_3$ . Solvent extraction APDC- chloroform. Atomic absorp- tion in isopropanol solution
	21.2	25.4	
	15.7	19.4	
1200/31/7		5.11	Direct with anodic stripping voltammetry
		5.45	
		11.89	

progress in oceanography, substantial improvement in the data will not be realized until oceanographic laboratories hire analytical chemists and an inter-laboratory comparison might be better predicated on this feature.

## VII. Current Needs

Much of the above has been directed toward illustrating less obvious aspects of the application of analytical chemistry to oceanography. The most pressing needs appear to be in the areas of determining the chemical species containing the various elements. Consideration of inorganic and organic complexing is popular but dependent on development of techniques and tools such as specific ion electrodes, gas chromatography and sensitive polarography. The micromolar concentrations of the in-

teresting compounds, both inorganic and organic, means that most techniques must be pushed near the useful limit. For example, specific electrodes for copper are not useful for the study of complexing in sea water because the copper concentration is too low. Synthetic laboratory work with copper is not practical because the many possible organic complexing agents have not been identified and synthetic systems would lack application to the real ocean. The most formidable and pressing need is the development of general use methods for organic compounds. We are not going to be able to understand the chemistry of the oceanic system until there are considerable advances in the data rate for organic species.

In terms of number of analyses, the biologically active elements will probably be dominant. Growth stimulation and toxicity are still only poorly documented for most of the metals and organic compounds. Even the long recognized nutrients are not adequately described, particularly the various nitrogen containing compounds. Usable but tricky methods for nitrate ion were developed several decades ago and the oceanic system has been described primarily in terms of the effects of nitrate. In the last decade, usable but very tricky methods for ammonium ion have been developed and the importance of this form in the nitrogen cycle and in algal nutrition has received increased attention. Recently, methods for urea have been developed and the first few papers show that this compound must be considered in describing the system quantitatively. Other forms of combined nitrogen may be important but are not considered because of a lack of analytical methods and methods development in this area would probably receive immediate application. Study of many of the compounds that are presently determined by bioassay, such as vitamins and metal complexing compounds, would benefit from the development of methods with improved precision, simplicity and speed of analysis.

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## PANEL DISCUSSION

### Analytical Problems in Oceanography



#### CHAIRMAN

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#### PANEL

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Theodore J. Kneip, Deputy Director, Laboratory for Environmental Medicine, New York University Medical Center, New York

James H. Carpenter, Professor of Earth and Planetary Science, Johns Hopkins University (Speaker)

**Bowen** — Currently at the Woods Hole Oceanographic Institution, chemicals that most people would consider pollutants are being used as tracers for oceanographic processes. For a long time, the artificial radioactivity from nuclear weapons test fallout has been used as a tracer. This type of approach is presently being expanded to using petroleum hydrocarbons and chlorinated hydrocarbons, and it is hoped to use some heavy metal pollutants as tracers for physical and geochemical studies of movement in the ocean.

In the course of the work with artificial radioactivity, one of the most serious needs for a standard reference sample was one that could be certified as a blank. This requirement came up over and over again. Samples of seawater consisting of 100 or more individual units were produced relatively easily. Each 60-liter unit of seawater was of uniform and measurable content of strontium-90, cesium-137, plutonium, *etc.* But, when it came to providing a seawater facsimile that one could be assured didn't have any of these constituents, serious problems were encountered. The blank sample finally obtained, easily cost 10 times more per unit than did the others.

On the other hand, in studies of this sort, and this is true whether one is motivated by pollution or tracer concern, it is almost always going to be necessary to make sections of analyses outward from a point source or downward from a known plane source to as close as possible to zero concentration. The only way a large number of different analysts can be assured that they mean the same thing when they say zero is by being provided with a blank sample, in much the same way as they are provided with a standard sample.

The group at Wood's Hole is now greatly concerned as to how they can do this with chlorinated hydrocarbons. It is now felt that they have some samples that are usable and are free from non-recently-biosynthesized petroleum hydrocarbons. Hope was expressed that in the Standard Reference Materials program of the National Bureau of Standards as is now being contemplated by a similar organization of the International Atomic Energy Agency, serious consideration will be given to the provision of blank samples. Standard Reference Materials certified for all of the major pollutants of present environmental concern, plus some that can be anticipated in the future, are needed. Help is being received from other agencies in the preparation of reasonably well-certified biological samples for chlorinated hydrocarbons. However, this takes a great deal of work, a great deal of expertise, a great deal of time, and a great deal of money. Yet all comparable analytical programs have shown this to be essential.

Another point that should be discussed is the question of what should



be analyzed, specifically in the context of environmental concern. We really don't know the constituents of any of the major environmental systems such as seawater, fresh water, river water, sediment, soils, *etc.* We are still in the first stage of Dr. Butler's sequence of increasing sophistication. We must continue to analyze all of these systems for the elemental constituents and must at the same time collect data, which is not widely being done, on inputs of new materials.

An interesting illustration is the present problem of the distribution of plutonium. It has been known for nearly two decades now that plutonium was being introduced into the environment, but it was believed that this was not going to be a matter of concern and we formed the habit of introducing it without determining how much was being introduced in any place at any time. It is now found that Pu is a material of considerable concern, about which a great deal must be learned quickly and in some cases rather dirtily. This information might very well have already been known if we had been willing to look a little further ahead.

At this point though, the analytical chemist really has to rely on somebody else to tell him which of these systems are going to be worth analyzing at any given time. In detail and extensively, he has to go to the biologist, the ecologist, the geochemist, and so on. These people will give him predictions which are based on data, but they are based on data as interpreted by value judgements. However, it often isn't going to be obvious, how extremely poor is the data on which much of this expression of concern is in fact based.

Although a considerable body of estimates of toxic levels exists for various things, there are virtually no instances where the estimates of toxic levels of trace elements are based on an actual measurement of toxic concentration in the system. Normally, the toxic level is described simply in terms of the amount that was added to a unit volume containing one or more organisms. It is now known that this can't be any more than an upper limit. It is also known that the rate at which an organism sees these things is always critical.

If a fresh water clam is exposed to only that amount of manganese which is contained at any unit time in his tissues and if this is presented to him, all at once, as manganous ion in solution, it will kill him and kill him quickly. On the other hand, the manganese which had accumulated over a long period of time was in a biochemical form of extreme stability and didn't bother him in the least. This is relevant to what was said this morning concerning mercury in some of the large fishes. They have accumulated this in the same sort of way, and it is, almost certainly, equally inert biochemically. When you do extrapolate from a level of concern by a

biochemist, you have to do this with some reservations and should put a little pressure back on the biologist to look more carefully.

The speciation problem which has been discussed for the past several days is a special instance of this. It isn't always obvious that we need to know the chemical species of toxic elements. In a considerable number of cases, it can be shown that the rate of the uptake process by organisms or of the geochemical removal process is slow relative to the rate of equilibration among the various physicochemical species involved. It can also be shown that the stability of the biological form or the geochemically immobilized form is high compared to these. In that case, all one needs to know is the total concentration because the total concentration will be effective in driving the reaction toward the combined form. It is necessary that we know in some detail the process with reference to which the measurements are to be made; otherwise the speciation question is of considerable academic interest from the physicochemistry of the system, but it can't be shown to be necessary to the environmentalist.

Some really weird, unforeseeable speciation problems are encountered. One has just come up in connection with a major intercalibration experiment being done under the auspices of the International Atomic Energy Agency. It was necessary for investigators from a large number of different countries to be able to produce analytical data on radioactive waste disposal, specifically from reactor wastes. It was decided that samples had to be collected, homogenized, and distributed for this purpose. We had a good deal of experience for doing this for comparable samples of fallout radioisotopes of the same elements. We knew what kind of containers were suitable, we knew the pH to which the solution had to be reduced, we knew how to take off those species which were adsorbed onto the walls of the containers, and we plugged all of this information into the experiment.

Scientists in Britain collected a large amount of rather highly radioactive water from the Irish Sea, put it into containers and distributed it. So far nobody is reported to have been able to get the radioactive cerium, the radioactive zirconium, or several of the other constituents out of the plastic containers by any treatment whatsoever. The material is known to be there because the solutions are hot enough, that we can set the whole two-liter container on a gamma spectrometer and see the peaks, but we can't get them out. Apparently the radionuclides in these samples are present in a series of chemical species which were completely unlike those in the fallout with which we were experienced.

There is one other very curious case which presumably must be related to speciation of some reasonably, abundant dissolved constituents. The

biogenous sediments on the ocean floor can be reasonably easily divided into two major classes, one is the calcium carbonate sediments and the other is the siliceous sediments consisting of diatoms or of radiolaria. Generally these two sediments do not coexist. Considerable areas are found where one or the other predominates. No measurable differences can be seen in the water which overlies one type of sediment from the water which overlies the other type, and yet a fairly major phenomenon is clearly different in the two cases. This is a very beautiful analytical problem and of considerable geochemical interest.

There are definite problems when one physically collects a sample a mile below sea level in a container that can withstand the pressure. There is a principle that is common to all such samplers: one lowers an open tube to the depth from which the sample is to come, then at depth one closes the tube, and brings the sample back. Presently the one absolutely unsolved disadvantage of this is that such a sampler sees all of the surface-active material between the surface and the point of collection, at which point it is closed. We have no way of obtaining large water samples or sediment samples that are not comparably contaminated by surface-active agents. We have built some rather large samplers for some other reasons, and it turns out that the larger the sampler, the smaller the ratio of surface to volume, and the better the sample is for almost any purpose, if the sampler is made of suitable material. It should be noted that several devices collect small samples of water at depth, for microbiological purposes, without "seeing" the overlying water.

If one asks what kind of sensors can be used at what depths, and how far can one go down with certain types of sensors, we know that salinity, temperature, radiation and density sensors can go down to all depths. The deepest I have seen an oxygen sensor used has been at about 1000 meters, some years ago. It is probably correct to say that there are no other sensors that can be used successfully at any significant depth at all. We expect this situation to change rapidly.

**Fassel** — The last sentence in Dr. Carpenter's abstract states that "For the trace constituents including many of the heavy metals, inaccuracy by current techniques and analysis has produced a nearly useless body of data." I am in complete agreement with Dr. Carpenter's conclusion that compositional characterization at the ppm, fractional ppm, or ppb levels poses singularly difficult problems, but what are the problems? They stem primarily from the fact that trace element technology is not a mature science. Analytical problems posed by oceanographic research serve as excellent examples to illustrate this immaturity.



It is appropriate to recall that all of the elements in the earth's crust are found in the ocean, but only 13 occur at concentrations above 1 ppm, and the majority are present at less than 1 ppb. In spite of the striking advances that have been made in trace element determinations, an analyst must still be extraordinarily successful to measure impurities at these concentrational levels with sufficient accuracy and precision to allow meaningful statistical evaluations and correlations that are so necessary to oceanographic studies. Even if the analyst develops the observational capability to achieve accuracy and precision at these concentration levels the problem is only partially solved. The taking of the sample and its handling prior to the analysis must also be factored in. The risks of intolerable contamination or the loss of significant impurities are present from time of sampling, during storage, and up to the final measurement.

Unfortunately, the various sources of contamination or the processes through which impurities may be lost (adsorption, incomplete separations, volatilization) have not been adequately identified, nor are they usually obvious. All too often the consequences of contamination or loss of impurities are ignored. The various pathways that contribute contamination or loss must be identified more adequately and controlled more effectively before trace element technology can be labeled a mature science.

Trace element technology is immature from another standpoint. Whether we are concerned with the role of trace elements in solid state science, or evaluating their biomedical effects, or assessing the degree of water or air pollution, or observing the transport of trace constituents in the ocean, there is a need for determining multiple trace elements in a large number of samples. This need can, in principle, be satisfied by spark source mass spectroscopy for many types of samples. However, the cost of the hardware and staff competency are often prohibitive. Moreover, the direct quantitative analysis of small amounts of liquid samples of the type often encountered in biomedical and health studies, is beset with many problems. Another popular way to perform multielement trace determinations is by neutron activation analysis, but here again the hardware expense may be prohibitive, the element coverage is somewhat limited, and for some elements the time required for irradiation may be unacceptably high. Presently the most popular technique for determining trace elements is flame atomic absorption spectroscopy (FAAS), but as presently practiced, FAAS is not a multielement technique. The determination of say thirty trace elements in an oceanographic water sample involves too many time-consuming mechanical operations.

There is needed, therefore an analytical technique that satisfies the following criteria: (a) the analytical operations should be reasonably simple



in their execution; (b) the hardware requirements should be met with relatively inexpensive instruments; (c) sample manipulation and treatment prior to analysis should be minimal; (d) simultaneous or rapid sequential multielement determinations should be possible; and (e) the power of detection of the technique should be in the part per billion range.

During the past six years we have explored an analytical system that satisfies the requirement stated above to a degree higher than any other analytical approach known to me. This technique relies on one of the oldest experimental methods for the determination of trace elements, namely, the observation of optical emission spectra, emitted not in conventional arc or spark discharges but by inductively coupled plasmas operated at atmospheric pressures. Since this technique and its potential advantages have been adequately described in the literature, I shall just remind you that the observed powers of detection of plasma emission compare favorably to and in many cases are vastly superior to the best atomic absorption values so far reported. The combination of this plasma with a direct-reading optical spectrometer provides the analyst with the capability of determining simultaneously as many elements as there are channels in the spectrometer.

**Juvet** — Analytical chemists should be able to contribute in this field by suggesting modernization of the measurement methods currently used from the buret-titration type to something of equal or better accuracy. For example, have coulometric methods for chlorinity been tried where the value of the faraday is very accurately known? Using instrumental methods such as this, where one has only to push a button and have the answer printed out, should be very desirable since shipboard conditions would be much less a factor in the results.

It would also seem possible using present day knowledge to design sensors to analyze these materials at depth rather than taking hours to bring them to the surface to be analyzed. Certainly analytical chemists could be of major assistance in the design of such instruments. Important instrumental design considerations which must be kept in mind in oceanographic studies include not only corrosion and erosion effects of the ocean and marine organisms but also the tremendous pressure encountered at the ocean depths. Pressure increases at the rate of about 1 atmosphere for every 33 feet of depth in seawater. Since the deepest known area in the ocean, the Mindanao Trench, has a reported depth of 37,782 feet, a pressure equivalent to well over  $16,000 \text{ lb}\cdot\text{in}^{-2}$  must be withstood. One published article was noted in which activation analysis was used to analyze the mineral content of the sea floor. In the case, the neutron

source was 200 micrograms of californium-252. The gamma rays produced by the irradiated sea floor indicated the elemental content.

**Kneip** — I would like to make a few remarks concerning the concentration of heavy, or transition, metals. Statements were made today and other days that the heavy metals become concentrated. Unfortunately, this statement is misleading. We have all learned that the pesticides are concentrated up through food chains to the point where the top predators are being lost because of this concentration effect. When someone says that the heavy metals are concentrated in the food chain, the immediate impression is that the same sort of phenomenon takes place; the higher one goes in the food chain, the higher the concentration.

By and large, this is not true for metals. In fact mercury is an exception because we are dealing with a heavy metal which is converted to a metal-organic compound and it concentrates in the food chain because of the specific transfer of the organic material. It is stored differently from the pesticide. There is a methylmercury-protein complex in muscles, in addition to storage like the pesticides in fat. Thus to use mercury as a representative example of heavy metal concentration factors in biological systems is misleading.

The Institute of Environmental Medicine of New York University Medical Center has been specifically and rather intensely looking at manganese and cobalt in the Hudson River estuary in the vicinity of a nuclear plant because of interest in the circulation of radionuclides that are released by that plant. There is a need to predict what will happen to future releases by additional plants being built in the region. The very first thing one finds out is that these transition metals are heavily concentrated by plants, and this includes plankton and rooted plants. The higher forms of life in the region, the fish for instance, do not have nearly as high a concentration of the transition elements as the plants. Not only this, but response times are vastly different. The plants are responding in very short time periods. These time periods have not been resolved but they are less than a week for rooted plants, while for the entire system, *i.e.*, waiting for the fish to come into equilibrium with everything else, will take anywhere from a month to two months.

The sedimentary interactions have been spectacularly important in understanding the system. It was found that manganese and cobalt for instance, regardless of their origin, transfer to the sediments while the water in the region is fresh, but when the salinity reintrudes into the area, huge quantities of manganese and cobalt are released into the water. The sediments are the only reservoir in the system containing sufficient

manganese to supply the amounts that appear in solution. There is an immediate response of the plants to this manganese. They take it up, concentrations jump, and the whole system reverses itself when another intrusion of fresh water comes through the region.

This is one of the confusion factors that adds to the fun in studies of the estuary. It is not the same from day to day and, if you plan your experiments for certain conditions and the weather doesn't cooperate, you can find yourself on a body of fresh water when you planned to have a certain salinity which is normal for that time of year.

It is fascinating to contemplate what is happening to some of the other heavy metals. All of these materials seem to go to the sediments, but whether all of them will behave in a similar fashion is doubtful. Multivalent states do not occur in many of the other metals. The chemistry of manganese is unique, so one may find that there is a storehouse for some of the heavy metals in this region rather than simply a transfer point. It would be interesting to know whether manganese itself is undergoing this cycling is merely temporarily held in the region and is carried out to sea as it is redissolved.

There are times and places when the team leader in these problems should be an analytical chemist. It is very obvious from some of the problems of understanding interactions of sampling, the sampling container, the need that originated the study, the analytical methods that are available, and finally the interpretation of the results to get back the answer to that original need, that the analytical man is the only one who is going to put it all together. He is able to take some of the information from one particular speciality and some from another, then supply the overall look at the whole problem and become the team leader. We should not become so intent upon being a service in this field. We ought to try to get into the heart of the problem and, where it is appropriate, take the lead.

**Carpenter** — The most exciting thing in this regard is that the flux of elements from the sediments is not entirely controlled by molecular diffusion. Profiles are seen that can only be interpreted by the bubbling of methane, motion of organisms, *etc.* So we are going to be stuck with the familiar problem of how to understand "turbulent diffusion" while looking at the flux of these metals from the sediments. In reference to the comment this morning about phosphate, a lot of phosphate is seen in the sedimentary record. Presumably there are chemical conditions where phosphate does accumulate, so that if those conditions were understood well enough, in some places where we would like the phosphate to remain

in the sediment, a way could probably be figured out rather than removing it all. Perhaps some creativity in the direction of understanding what should be done if we don't want the phosphate to come out might be very helpful.

In regard to sampling at great depths, one ambitious group has pumped samples from several miles. The problem is that you sit there and try to convince yourself that the sample does not change with time, and you begin to wonder about sample integrity. However, with expenses of several thousand dollars a day for the ship, one gets rather impatient sitting there trying to make up his mind whether the whole sampling apparatus has come to equilibrium yet.

If we ask whether there are any geological data on which one can establish a baseline in the sea deposit, such as saltbeds that could be used to determine the history of trace elements in seawater, the answer is yes, at least to a useful approximation. Most of the data we have, unfortunately, were collected by a few people who chose emission spectrographic methods and then tested them against other methods with considerable frustration. There were factors of 3 or 4 differences. So to that degree, to factors of about 5, samples from sediments of all the world's oceans have been analyzed. We have some ideas of sedimentation rates in these areas, so one can make an approximate geochemical balance that is probably not uncertain by more than a factor of 5.



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